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Variation in and adaptive plasticity of flower size and drought-coping traits

Susan C. Lambrecht · **Aggie Morrow** · **Rachel Hussey**

Abstract Although phenotypic plasticity of morphological and physiological traits in response to drought could be adaptive, there have been relatively few tests of plasticity variation or of adaptive plasticity in drought-coping traits across populations with different moisture availabilities. We measured floral size, vegetative size, and physiological traits in four field populations of *Leptosiphon androsaceus* (Polemoniaceae) that were distributed across a rainfall gradient in California, USA. Measurements were made over 5 years that varied in precipitation. We also conducted a growth chamber experiment in which half-sibs from three populations were divided equally among a well-watered and a drought treatment. We tested for selection on traits in each of the watering treatments, and evaluated whether traits exhibited plasticity. In the field, plant traits exhibited substantial variation across populations and years. Flower size, leaf size, and water-use efficiency (WUE) were generally higher for populations that received greater average rainfall. However, in dry years, we observed a decrease in flower and leaf size, but an increase in WUE across the populations. In the growth chamber experiment, leaf and physiological traits exhibited

plasticity, with smaller leaves and higher WUE found in the drought, as compared to the well-watered treatment. Only specific leaf area exhibited differentiation in plasticity among populations. Although there was no observed plasticity in floral size, selection favored smaller flowers in the drought treatment and larger flowers in the well-watered treatment. Our results suggest that moisture availability has led to trait variation in *L. androsaceus* via a combination of selection and phenotypic plasticity.

Keywords Floral traits · *Leptosiphon androsaceus* · Morphological traits · Polemoniaceae · WUE

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Introduction

Phenotypic plasticity is an important mechanism by which sessile organisms cope with changes in the environment around them. Through plasticity, plants may exhibit varying morphological, physiological, or life history traits under different environmental conditions (Bradshaw 1965; Via and Lande 1985). Plasticity has received much attention, not only for its role in enabling plants to persist in and adapt to heterogeneous environments, but also because it may be a key factor in enabling plants to cope with climate change and undergo range shifts (Ghalambor et al. 2007; Matesanz et al. 2010; Anderson et al. 2012). However, while it is often assumed that plasticity is

adaptive, its adaptive value is not always clear (van Kleunen and Fischer 2005; Valladares et al. 2006; Matesanz et al. 2010; Nicotra and Davidson 2010).

Water is a vital resource that is both spatially and temporally heterogeneous. Plants exhibit considerable plasticity in many different types of traits, alone or in combination, which enable them to limit water loss or avoid the effects of drought. Morphologically, several adjustments may be made. Reducing leaf size and number enables plants to limit the surface area from which water may be lost (Sultan and Bazzaz 1993; Gianoli 2004; Gianoli and Gonzalez-Teuber 2005; Couso and Fernandez 2012). Moreover, reductions of specific leaf area (SLA), or leaf surface area per unit mass, leads to smaller, thicker leaves, which can increase foliar resistance to water loss (Gianoli 2004; Caruso et al. 2006; Agrawal et al. 2008). Plasticity in stomatal density aids in the regulation of water loss (Xu and Zhou 2008; Fraser et al. 2009; Maherali et al. 2010), while an increase in root number or size facilitates water uptake and/or storage (Sultan and Bazzaz 1993; Heschel et al. 2004; Gianoli and Gonzalez-Teuber 2005; Mal and Lovett-Doust 2005). Physiologically, plants may exhibit plasticity in stomatal conductance, whereby they close their stomata to limit water loss under drought, increasing instantaneous water-use efficiency (WUE), which is the ratio of carbon gained via photosynthesis to water lost via transpiration (Heschel et al. 2004; Caruso et al. 2006; Sherrard and Maherali 2006; Nicotra et al. 2007; Maherali et al. 2010; Lázaro-Nogal et al. 2015). Plasticity in integrative WUE has been observed using stable carbon isotopes (δ^{13}C; Aspelmeier and Leuschner 2004; Franks 2011; Edwards et al. 2012; Kenney et al. 2014), which are often more reliable than instantaneous measures, because δ^{13}C reflects WUE integrated over longer periods of time. Finally, plasticity in development rate is an important mechanism for some plant species, such as annuals, to cope with drought by enabling them to reproduce and senesce before the onset of drought undermines their fitness (Gianoli 2004; Heschel and Rignios 2005; Sherrard and Maherali 2006; Maherali et al. 2010; Franks 2011; Kenney et al. 2014; Gugger et al. 2015).

While it is clear that many plants exhibit plasticity in response to moisture availability, the adaptive role of this plasticity is less clear (Nicotra and Davidson 2010). Theory predicts that resource heterogeneity will affect the evolution of plasticity in that

adaptiveness and, therefore, the frequency of plasticity will increase with environmental heterogeneity (Via and Lande 1985; Donohue et al. 2000; Baythavong 2011). In particular, when dispersal distances are smaller than the scale of environmental heterogeneity, adaptive plasticity is expected to be favored, whereby phenotypes respond to their local environment (Baythavong 2011). Support for the concept of adaptive plasticity in relation to moisture availability is limited and inconsistent. For example, some studies have shown increased levels of adaptive plasticity in drought-coping traits in populations receiving more heterogeneous precipitation, as compared with those receiving more consistent precipitation (Gianoli 2004; Gianoli and Gonzalez-Teuber 2005; Lázaro-Nogal et al. 2015). In contrast, Sultan and Bazzaz (1993) and Heschel et al. (2004) studied several populations of the generalist annual *Polygonum persicaria* (Polygonaceae) that experienced dissimilar moisture regimes. One population was consistently mesic, while the others experienced variably dry conditions. They found that levels of adaptive plasticity for morphological and physiological traits in response to moisture availability were similar across the populations. Similarly, Schlichting and Levin (1990) found similar levels of plasticity in vegetative and floral traits among seven populations of *Phlox drummondii* (Polemoniaceae) in response to moisture treatments, in spite of different annual average precipitation between the populations. These findings suggested that variation in moisture in both space (*e.g.,* heterogeneous moisture availability within a population) and time (*e.g.,* large episodic droughts occurring in some years) elicit the same type of response in plants, and may both maintain plasticity for drought-coping traits. Finally, some studies have found evidence that plasticity in relation to drought may actually be maladaptive. For example, Dudley (1996a, 1996b) found selection for increased WUE in *Cakile edentula* (Brassicaceae) growing in dry conditions. However, plants growing in these dry conditions had lower WUE than those in moister conditions, suggesting the direction of plasticity was opposite that favored by selection. These results may have arisen due to the underlying genetic correlations between WUE and other traits, such as leaf size (Dudley 1996a).

In addition to drought-coping traits, floral size also varies with moisture availability. Within species, plants in dry locations tend to produce smaller flowers

than they do in wetter locations (Herrera 2005; Lambrecht and Dawson 2007; Elle et al. 2010; Suárez et al. 2011; Lambrecht 2013). Although floral size tends to be less plastic than vegetative or physiological traits, reductions in flower size in response to decreased soil moisture availability have been demonstrated in several species (Carroll et al. 2001; Elle and Hare 2002; Mal and Lovett-Doust 2005; Caruso 2006; Edwards et al. 2012). This floral plasticity may be functional for plants. Given that flowers transpire significant amounts of water (Patiño and Grace 2002; Feild et al. 2009; Lambrecht et al. 2011; Lambrecht 2013; Teixido and Valladares 2014) and this water loss can affect leaf functioning, particularly under dry conditions (Galen et al. 1999; Lambrecht and Dawson 2007; Lambrecht 2013), variation and plasticity in floral size may be an important mechanism to control water loss when plants are faced with drought (Galen et al. 1999; Caruso 2006; Lambrecht and Dawson 2007). Investigating the adaptive role of floral size variation and plasticity in conjunction with leaf morphology, physiology, and reproductive traits may provide insight into the evolution of plants in relation to moisture availability (Edwards et al. 2012).

The objective of this study was to look at variation in plant traits of the annual *Leptosiphon androsaceus* Benth. (false babystars, Polemoniaceae) in response to moisture availability in the Mediterranean climate of California. Speciation within the genus *Leptosiphon* may be tied to the development of the summer dry Mediterranean climate in California (Raven and Axelrod 1978; Bell and Patterson 2000), suggesting that moisture availability has played an important role in the evolution of this genus. This species is distributed throughout California. Its growing season coincides with the annual winter rains, the duration of which varies considerably across years, leading to periodic droughts. In a field study, we examined variation of traits of the self-incompatible *L. androsaceus* across four populations distributed along a naturally occurring precipitation gradient to observe how traits vary over space and time. Our previous work with a highly selfing *Leptosiphon* species revealed significant variation in morphology and physiology over the same region used in this study (Lambrecht 2013). Field data for this study were collected over five years that varied substantially in precipitation, enabling us to examine trait variation

within and across populations over time. This trait variation may be a result of selection on traits or the result of plasticity. Therefore, we used a growth chamber experiment, in which seeds originating from three of the field populations were grown under two watering treatments (well-watered and drought) in order to test for the presence of trait plasticity and evaluate selection on trait means. In this study, we asked the following specific questions: (1) How do vegetative, floral, and leaf physiological traits vary across space and time in relation to moisture? (2) Is any variation in these traits due to plasticity? (3) Is there differing selection on traits in response to moisture availability?

Materials and methods

Study species

Leptosiphon androsaceus (formerly *Linanthus*) is a winter annual native to the western United States. In California, its seeds germinate with the onset of winter rains. It flowers in spring (April–May), and sets seed and senesces with the onset of the summer drought (May–June). It bears several flowers from a terminal head, each flower lasting up to 1 week. It is completely self-incompatible and pollination is primarily by long-tongued flies (Goodwillie 1999; Goodwillie and Ness 2013; Lambrecht unpublished data). Although precise dispersal distances are unknown, seeds are small and are not wind dispersed, so they do not travel far beyond the maternal plant.

Field sites and abiotic data

All field sites were located in Henry W. Coe State Park (Coe; ~35 000 ha), near Morgan Hill, California (Table 1). Several mountain ridges cross Coe in a north–south direction, creating a noticeable precipitation gradient from the western to the eastern side of the park. Four similar populations, which were at least 1 km away from one another, were selected along this gradient for study. From west to east, these populations were called Bobcat, Domino, Woodpecker, and Mustang (Table 1). All populations were in partially open oak woodlands or chaparral, had numerous (>100) individuals of *L. androsaceus*, a sandy loam soil texture as

Table 1 Field populations of *Leptosiphon androsaceus* listed from west to east

Population	Latitude, longitude	Elevation (m a.s.l.)	Average soil moisture (%)
Bobcat	37° 7.620′N, 121° 27.144′W	750	12.4 (0.9)[a]
Domino	37° 7.272′N, 121° 26.883′W	721	10.9 (0.5)[a]
Woodpecker	37° 9.716′N, 121° 23.707′W	459	9.3 (0.3)[b]
Mustang	37° 10.681′N, 121° 22.308′W	554	9.2 (0.7)[b]

Average soil moisture (1 SE) is the average moisture measured within each population during the growing season (April–June) of the 5 years of field study. Means of soil moisture that share a common letter are not statistically different ($P > 0.05$), according to the Tukey HSD pairwise comparison

determined by field textural analysis (Thein 1979), and similar soil nutrient levels.

Measurements of weather and soil conditions were made onsite and at nearby meteorological stations. During flowering, volumetric water content of the soil was measured weekly over the rooting depth of *L. androsaceus* (to 10 cm) in 3–5 permanently marked locations in each site with a time-domain reflectometry probe (Field Scout TDR 200, Spectrum Technologies, Plainfield, Illinois). Average seasonal values were calculated for each measurement location. Hobo ProTemp temperature loggers (Onset Computer Corporation, Bourne, Massachusetts) recorded temperature and relative humidity hourly throughout the growing season in each of the populations. These data were used to estimate precipitation for each population using multiple linear regressions, which were developed using daily average temperature, humidity, and precipitation measured at the meteorological station nearest to each population (~5–10 km; CIMIS 2012; WRCC (2012).

Field study

The field study was conducted from 2005 to 2011 (excluding 2009 and 2010). Each year, measured plants were selected at random from those that had receptive stigmas and were >0.5 m from other measured plants. Between 15 and 30 plants were measured in each population every year (total $n = 387$ plants).

We measured several morphological traits of *L. androsaceus*. Floral traits were measured with a digital caliper to the nearest 0.01 mm. Between 1 and 3 flowers were measured per plant, and average plant values were used in analyses. Measurements included the width and length of each of the five corolla lobes,

the diameter of the corolla face, and the length of the corolla tube. The average corolla lobe width and length were calculated for each flower. The stigma-to-anther distance (measured in 2007, 2008, and 2011) was measured as the distance from the central point of the stigma to the anthers. Measured vegetative traits included calyx length, leaf length, plant height from the ground to just below the terminal head (measured with a ruler to the nearest 0.1 cm), and total number of leaves.

To determine whether larger flowers received more pollen from pollinators, stigmas were collected from ten open flowers in each of the field populations in 2008 and were preserved on slides with fuchsin dye (Kearns and Inouye 1993). Stigmas were also collected from unopened flowers as a control to account for any self-pollen that may have collected on stigmas prior to opening. Slides were returned to the lab and pollen grains were counted under a microscope.

A subset of measured plants was collected each year for leaf area and carbon isotope analyses. In 2005 and 2011, five plants were collected from each population, while 10 plants were collected in each of the remaining years (total n over 5 years = 160). Leaf area of the uppermost pair of leaves was measured in the field using a portable leaf area analyzer (CID 202; CID Analytical, Camas, Washington). These areas, along with the caliper measurements of leaf length, were used to develop regressions to predict leaf area for uncollected plants. Collected plants were kept in a cooler until they could be returned to the lab and dried in a 60°C oven for 48 h.

To estimate WUE, stable carbon isotope ratios ($\delta^{13}C$) were measured for the collected plants. Carbon isotope ratios quantify $^{13}C/^{12}C$ of leaf tissues relative

to that of a standard (PeeDee belemnite; Dawson et al. 2002). Changes in stomatal conductance (g_s) can alter the ratio of $^{13}C/^{12}C$. Plants that close their stomata more often will have more ^{13}C in their leaf tissues, reflecting higher integrative WUE, than those plants that have their stomata open. Due to the calculation of $\delta^{13}C$ using standards, values are negative, with higher values (less negative) indicating higher WUE (Dawson et al. 2002). Plants were often too small for analysis without including stem tissue in the analysis, so stem and leaf tissues were ground to a fine powder using a ball grinder. In our previous work with this and a closely related species, we have found that inclusion of stem tissue does not significantly affect $\delta^{13}C$ values (Lambrecht unpublished data). In 2005–2007, a 4.0 mg (± 0.2 mg) subsample of ground material was analyzed for $\delta^{13}C$ using a PDZ *Europa Scientific 20/20* isotope ratio mass spectrometer interfaced with an ANCA-SL elemental analyzer (Northwich Cheshire, UK) at the Center for Stable Isotope Biogeochemistry (University of California, Berkeley, CA). Samples from 2008 to 2011 were measured using a Delta-V Advantage isotope ratio mass spectrometer with a Costech elemental analyzer at the Facility for Stable Isotope Mass Spectrometry (FIRMS, University of California, Riverside, CA).

Statistical analyses of field data

All statistical analyses were done with SPSS (v. 24.0, IBM Corp, Armonk, NY). To test whether soil moisture varied over space and time, we used two-way analysis of variance (ANOVA) using population, year, and population × year as factors. All measured plant traits were analyzed with multivariate analysis of variance (MANOVA), with both population, year, and population × year as factors. When significant effects were detected, we followed with individual ANOVAs for each dependent variable. We used Holm's sequential Bonferroni correction to adjust *P* values of these sequential analyses (Holm 1979). Nonsignificant interaction terms were dropped from the final models. For those traits exhibiting significant differences across populations ($P < 0.05$), we used the Tukey HSD test to make pairwise comparisons of populations. We also used Pearson correlations to examine the relationship between average annual soil moisture per site and average annual values for each trait. For these and all further statistical analyses, the assumption of normality for each trait was tested using the Kolmogorov–Smirnov test. Visual examination of the residuals was used to assess homogeneity of variances. Variables that did not conform to model assumptions were log transformed (height) or square-root transformed (stigma–anther distance and corolla tube length) so that model assumptions were met.

We used Pearson correlations to examine the relationship between flower size (corolla lobe length, width, and corolla diameter) and the number of pollen grains received. When significant correlations were found, analysis of covariance (ANCOVA) was used to compare the relationships between flower size and pollen delivery across the populations. Separate models were run for each of the three parameters of flower size. Tukey HSD post hoc comparisons were used to compare populations.

Growth chamber experiment

A growth chamber study was used to test for the presence of plasticity in several plant traits. Seeds were collected in the field in 2011 from six maternal plants in each of three populations (Bobcat, Domino, and Woodpecker). We were unable to obtain seeds from the Mustang population for this experiment. To initiate germination, ten seeds per maternal line were placed on moist Kimwipes, with separate petri dishes for each line. The dishes were placed in a refrigerator at 2 °C until germination (9–11 days). There was sufficient germination from 4 maternal families (6–8 seeds each) per population to be included in the experiment (total $n = 88$). Germinated seedlings were then transferred to 10 cm tall conetainers (Stuewe and Sons, Tangent, Oregon) filled with well-watered potting soil mix (Sunshine Mix #5, Sun Gro Horticulture, Vancouver, Canada). The bottoms of the conetainers were placed in water reservoirs so that plants were bottom-watered for the duration of the experiment, with the exception of when fertilizer (10 mL of a 0.5% solution of 20-20-20 fertilizer) was added after about 2 weeks. All seedlings were transferred to two growth chambers (Conviron, Winnipeg, Canada), with half of the seedlings from each maternal line in each chamber. The chambers were set to a 12 h photoperiod, with a daytime temperature of 25 °C and a nighttime temperature of 12 °C. For the first week, all seedlings were misted from above, and water was added to a height of 3.5–4 cm in all

reservoirs ∼ every 2 days, until the seedlings were established. After 1 week, the watering treatments were initiated. Within each chamber, half of the seedlings from each maternal line were randomly assigned to the "well-watered" treatment, while the other half were assigned to the "drought" treatment. Plants and treatments were randomly arranged within each of the chambers, with plants in the same treatment sharing reservoirs. The plants within a treatment and the location of the four reservoirs were changed twice during the experiment. The water height in the reservoirs of the "well-watered" treatment plants was maintained at a height of 3.5–4 cm, with water added ∼ every 2–3 days. The drought treatment plants received no further water in their reservoir. This dry-down treatment reflects what happens in the field when rains end early in the growing season. In a preliminary experiment, we found plants were able to grow several months after watering ceased (Lambrecht unpublished data).

After 10 weeks, several morphological and physiological traits were measured on the plants. Corolla lobe width, length, corolla diameter, floral tube length, and calyx length were measured in the same manner as in the field, during peak flowering. Total number of leaves were counted for each plant. Measurements of instantaneous photosynthesis (A) and stomatal conductance (g_s) were measured using a LI-6400 XTR (Li-Cor Biosciences, Lincoln, Nebraska). All measurements were made between 900 and 1300 h PST using an attached red-blue LED set to a constant 800 μmol m^{-2} s^{-1}. During measurements, [CO_2] was held at a constant 380 μmol m^{-2} s^{-1} and vapor pressure deficit varied between 1.0 and 1.9 Pa. Because leaves were so small, we placed several in the chamber along with stems to facilitate the measurements, because the stems are also photosynthetic. After gas exchange measurements were made, leaves and the stem that were inside the photosynthetic chamber were collected and measured using the CID portable leaf area meter. Photosynthetic measurements were then area-corrected for the amount of photosynthetic tissue inside the chamber. Leaves were then removed from stems, and the area of leaf tissue alone was measured. The leaves and stems were separately placed in a drying oven at 60 °C for 48 h, and leaf dry weights were measured. SLA was calculated as leaf area per unit leaf mass. Leaf and stem tissue were then combined and prepared for δ^{13}C

analysis, as was done with field-collected plants. These analyses were performed at the Facility for Stable Isotope Mass Spectrometry (FIRMS, University of California, Riverside, CA).

Statistical analyses of growth chamber data

To determine if the measured traits exhibited plasticity in response to the moisture treatment, we used two-way ANOVA, with population of origin, watering treatment (wet vs. dry), and the interaction between the two used as factors. Block (growth chamber) was not significant, and was not included in the final models. A significant treatment effect indicated that the trait exhibited plasticity in response to the moisture treatment, while a significant interaction indicated the populations responded differently to the moisture treatment. We ran these analyses as univariate tests, rather than multivariate tests, to avoid complications when simultaneously analyzing regressions for highly correlated traits (Mitchell-Olds and Shaw 1987). We adjusted P values with the Holm's Bonferroni correction. Variables that did not initially meet model assumptions were log transformed (corolla tube length, photosynthesis, and stomatal conductance) to satisfy assumptions.

We used linear regression to test for selection on each trait in each of the treatments. For these analyses, we used leaf number per plant as the measure of fitness, because there could be no seed set in the pollinator-free growth chambers, and because leaf number will impact fitness via photosynthetic carbon gain (Caruso et al. 2006; Donovan et al. 2007; Nicotra et al. 2007). Moreover, leaf number was highly correlated with seed number and seed mass for *L. androsaceus* in the field ($r > 0.70$, $P < 0.001$, $n = 63$). These plants produce pairs of leaves at regular intervals along their stems, so leaf number is an indicator of plant size. For each plant, we calculated a relative fitness as the leaf number relative to the overall average leaf number. We calculated standardized values (mean = 0, standard deviation = 1) for each of the measured traits. We then ran separate linear regressions for each combination of treatment and trait between relative fitness and each standardized trait, including "population" as a fixed term and "maternal" line as a random term in the models (Gianoli and Gonzalez-Teuber 2005).

Results

Abiotic conditions

Populations differed significantly in precipitation and soil moisture. Precipitation declined across the populations, from west to east (Fig. 1). The 5 years of this study varied broadly in overall precipitation (across all populations, average annual rainfall in 2005 = 500 mm, 2006 = 440 mm, 2007 = 329 mm, 2008 = 285 mm, and 2011 = 427 mm). Soil moisture also varied across populations ($F_{3, 68} = 12.17$, $P < 0.001$) and years ($F_{4, 68} = 22.49$, $P < 0.001$), in a pattern similar to that of precipitation (Table 1). There was a significant population × year interaction ($F_{12, 68} = 4.74$, $P < 0.001$); the two populations with the greatest soil moisture availability (Bobcat and Domino) exhibited more inter-annual variation in soil moisture than the two drier populations (Woodpecker and Mustang).

Field study

Plant morphological traits varied across populations and years (Table 2). The different parameters of flower size differed by 10–24% across the populations. Correlations between average soil moisture and average trait values were positive ($0.45 < r < 0.66$, $0.001 < P < 0.05$), with the exception of corolla tube length ($r = 0.17$, $P = 0.47$), stigma–anther distance ($r = 0.53$, $P = 0.08$), and WUE ($r = 0.35$,
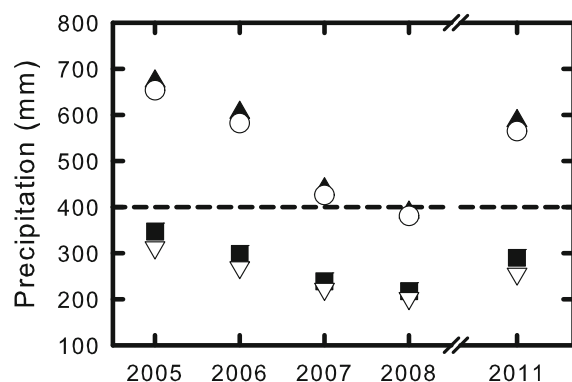
Fig. 1 Annual precipitation in each of the populations during the 5 years of field study. *Symbols* indicate the population, where the *filled triangle* is Bobcat, the *circle* is Domino, the *square* is Woodpecker, and the upside down *triangle* is Mustang. The *reference line* indicates the average precipitation in Coe over the period 1975–2010

$P = 0.13$). Following the Holm's Bonferonni correction, the floral traits that varied included corolla diameter ($P < 0.001$), corolla lobe width ($P < 0.001$; Fig. 2a), corolla lobe length ($P < 0.001$), and calyx length ($P < 0.001$). Leaf area ($P < 0.001$; Fig. 2b) and plant height ($P < 0.001$) were greatest for plants from the populations receiving the most precipitation and having the highest soil moisture content. All morphological traits exhibited significant variation across years ($P < 0.001$). There were significant population × year interactions ($P < 0.001$) for corolla lobe length, corolla diameter, corolla tube length, and plant height following the Holm's Bonferroni correction. Traits were larger in wetter (*e.g.*, 2005, 2011) than drier (*e.g.*, 2007) years.

Physiologically, integrative WUE differed across populations ($P < 0.001$; Fig. 2c and Table 2). The population × year interaction was significant only before the Holm's Bonferroni correction. According to Tukey HSD comparisons, plants in Mustang, the driest population, exhibited similar WUE to plants in Bobcat, the population with the highest soil moisture. WUE also varied across years ($P < 0.001$), with higher WUE in drier years.

Pollen counts were significantly higher on stigmas of larger flowers, suggesting pollinator preference for larger flowers. Regardless of the measure of flower size used (width or length of corolla lobes, diameter of corolla), the amount of pollen on stigmas increased with increasing flower size ($0.36 < r < 0.45$, $P < 0.02$). There was no significant difference in this pattern among populations ($0.36 < F_{3,32} < 0.53$, $0.67 < P < 0.79$). Populations did differ, however, in the amount of pollen delivered ($F_{3,35} = 4.28$, $P = 0.01$). The two populations receiving the most rainfall had significantly more pollen grains per stigma (Bobcat = 232.4 ± 23.2 pollen grains, Domino = 355.7 ± 77.9 pollen grains) as compared to the two drier populations (Woodpecker = 123.2 ± 22.9 pollen grains, Mustang = 82.9 ± 11.0 pollen grains).

Growth chamber experiment

Several measured plant traits exhibited significant plasticity in response to the watering experiment (Table 3; Fig. 3). In particular, vegetative and physiological traits responded to water availability, with plants in the drought treatment exhibiting traits more

Table 2 Average values (1 SE) for morphological and physiological traits across four field populations of *Leptosiphon androsaceus*

Trait	Treatment means				F-statistics		
	Bobcat	Domino	Woodpecker	Mustang	Population[1]	Year[2]	Pop × year[3]
Corolla lobe width (mm)	5.7 (0.1)[a]	5.6 (0.1)[a]	4.9 (0.1)[b]	4.6 (0.1)[c]	<u>56.85***</u>	<u>10.65***</u>	2.09*
Corolla lobe length (mm)	8.9 (0.1)[a]	9.1 (0.1)[a]	7.7 (0.1)[b]	7.6 (0.1)[b]	<u>56.55***</u>	<u>4.59***</u>	<u>5.05***</u>
Corolla diameter (mm)	18.3 (0.3)[a]	18.7 (0.3)[a]	16.7 (0.2)[b]	15.8 (0.3)[c]	<u>30.50***</u>	<u>5.44***</u>	<u>3.38***</u>
Corolla tube length (mm)	18.5 (0.2)	18.7 (0.3)	19.2 (0.3)	17.9 (0.3)	1.73	<u>22.24***</u>	<u>5.26***</u>
Stigma–anther distance (mm)	2.3 (0.1)	2.2 (0.2)	1.8 (0.1)	1.9 (0.1)	2.28	<u>10.67***</u>	NS
Calyx length (mm)	8.2 (0.1)[a]	8.6 (0.1)[a]	7.3 (0.1)[b]	7.4 (0.1)[b]	<u>14.13***</u>	<u>3.85***</u>	1.83*
Leaf area (cm²)	0.41 (0.03)[a]	0.41 (0.04)[a]	0.21 (0.02)[b]	0.17 (0.01)[b]	<u>21.17***</u>	<u>9.14***</u>	1.87*
Height (cm)	18.9 (0.6)[a]	19.1 (0.6)[a]	16.9 (0.7)[b]	15.8 (0.7)[b]	<u>14.03***</u>	<u>84.65***</u>	<u>3.70***</u>
Integrative WUE (δ¹³C, ‰)	−29.25 (0.14)[a]	−28.72 (0.15)[b]	−30.43 (0.16)[c]	−29.37 (0.19)[a]	<u>22.60***</u>	<u>11.57***</u>	2.08*

All measures were made during the five years of the study, with the exception of stigma–anther distance, which was measured in 3 years. For those traits for which there was a significant difference across sites, Tukey HSD pairwise comparisons were made. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Underlined values were significant following the Holm's Bonferroni correction of P values. An NS for the interaction term indicates that this term was not significant, and was removed from the model. Means that share a common letter are not statistically different ($P > 0.05$)

[1] Population df = 3367, except for stigma–anther distance (df = 3250) and $\delta^{13}C$ (df = 3128)

[2] Year df = 4367, except for stigma–anther distance (df = 2250) and $\delta^{13}C$ (df = 4128)

[3] Population × year df = 12,367, except $\delta^{13}C$ (df = 12,128)

consistent with water conservation (lower SLA, shorter calyx, lower photosynthetic and stomatal conductance rates, higher WUE), as compared with plants in the high moisture treatment (Holm's Bonferroni $P < 0.035$). Floral traits other than calyx length, however, did not exhibit plasticity in response to moisture availability (Holm's Bonferroni $0.35 < P < 0.99$; Table 3).

Populations differed in some drought-coping traits, but were similar in their response to the drought treatment (Table 3; Fig. 3). Only SLA exhibited a significant treatment × population interaction, indicating that the populations responded differently to the watering experiment (Holm's Bonferroni $P = 0.036$). SLA for both Bobcat and Domino plants was ~40% lower in the drought treatment than in the wet, indicating that drought treatment plants from these two populations may have had thicker leaves than well-watered plants (pairwise treatment comparison for Bobcat $P = 0.001$, for Domino $P = 0.02$). Woodpecker plants always maintained low SLA in both

treatments (pairwise treatment comparison $P = 0.26$), and had significantly lower values than the other two populations in the well-watered treatment (pairwise population comparisons in the well-watered treatment $P \leq 0.001$).

Populations did not differ in flower size. Although corolla lobe width and corolla diameter were different before the Holm's Bonferroni correction ($P < 0.05$), they were not significantly different after the correction.

For the traits exhibiting plasticity in response to the watering experiment (*i.e.,* significant treatment effect), there was selection for increased stomatal conductance and photosynthesis in the wet treatment ($\beta = 0.12$, $P = 0.05$ and $\beta = 0.17$, $P = 0.05$; Table 4). There was no selection for any other trait that exhibited plasticity in the drought experiment (calyx length, SLA, and WUE). Although flower size traits did not exhibit plasticity, there was selection for larger corolla lobe width and length in the well-watered treatment, and for smaller values in the drought treatment (Table 4).
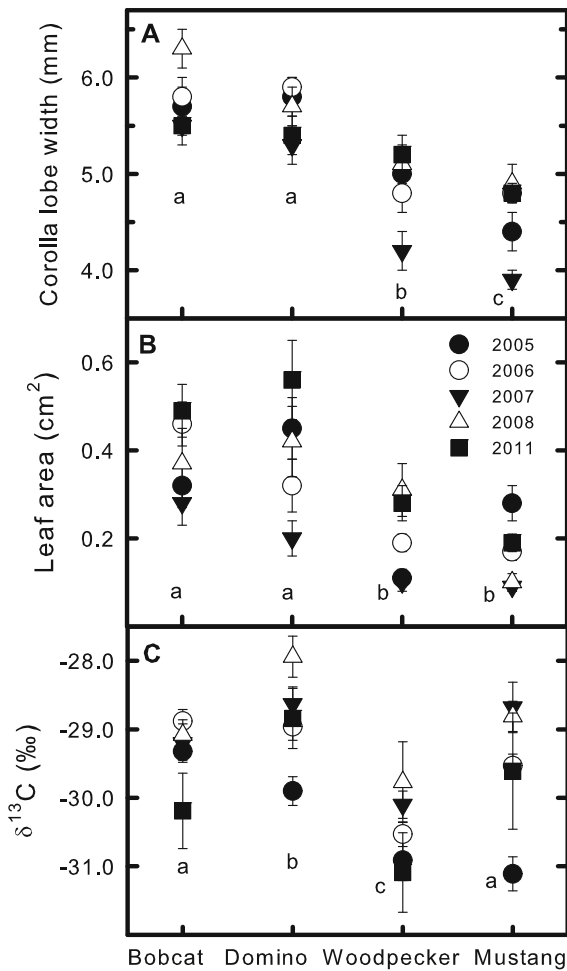
Fig. 2 Average plant traits (1 SE) in each of the populations during the 5 years of field study. **a** Average corolla lobe width (mm), **b** leaf area of the upper pair of leaves (cm^2), and **c** integrative WUE (δ^{13}C, ‰) in each of the five years of study. For each trait, populations sharing a common letter have statistically similar means ($P > 0.05$) across years based on the Tukey HSD pairwise comparison

Discussion

In our study, we examined variation and plasticity in plant traits in response to moisture availability by conducting a 5-year field study of four populations of *Leptosiphon androsaceus* situated along a naturally occurring precipitation gradient, along with a growth chamber experiment in which moisture availability was manipulated. In the field, we found substantial variation across populations and years. The spatial and temporal patterns of morphological traits were similar in that plants were larger and produced larger leaves

and flowers in wetter years and locations. Our growth chamber experiment identified significant plasticity in vegetative and physiological traits, but limited selection on these traits. Floral size traits, on the other hand showed no plasticity, but selection indicated that flower size should decrease under drought.

Is variation in drought-coping traits adaptive?

For annual species like *Leptosiphon androsaceus*, which grow in regions and habitats experiencing ephemeral moisture availability, strategies for coping with drought are vital to their fitness. In response to our drought treatment, plants increased WUE and decreased both SLA and stomatal conductance, all of which promote water conservation and drought tolerance. In the field, plants also increased WUE in drier years. Of these drought-coping traits, we only found selection for increased stomatal conductance in the well-watered treatment.

Studies of the variation, plasticity, and selection for WUE have produced varying results. While some studies have found selection for increased (Dudley 1996a, 1996b; Heschel et al. 2004; Nicotra et al. 2007) or decreased (Donovan et al. 2007; Franks 2011; Kenney et al. 2014) WUE under drought, others have found no indication of selection (Maherali et al. 2010; Ivey and Carr 2012). Moreover, WUE responds differently to various drought regimes. For example, Heschel and his colleagues (Heschel et al. 2002; Heschel and Rignios 2005) found that the direction of selection for WUE changed in *Impatiens capensis* (Balsaminaceae) depending on the onset of drought, where early season drought favored low WUE, while late season drought favored high WUE. Similarly, Wu et al. (2010) found that various drought treatments (*i.e.,* gradual dry down vs. consistent low moisture availability) affected SLA differently, although the direction and magnitude of the effect varied across the species of *Mimulus* (Phrymaceae) investigated in their study. Our drought treatment in the growth chamber was similar to what would happen if rains stopped early in the growing season, but there are other patterns of precipitation that may lead to drought in our field populations. Thus, varying patterns of precipitation may have produced the spatial pattern of WUE we observed, with higher WUE in wetter populations, in spite of the increase in WUE we observed in drought years and in our drought

Table 3 Phenotypic data of *Leptosiphon androsaceus* measured in a growth chamber experiment in which water availability was manipulated

Trait	Treatment means		F-statistics		
	Well-watered	Drought	Treatment[a]	Population[b]	Treatment × population[c]
Corolla lobe width (mm)	4.5 (0.1)	4.3 (0.1)	2.12	3.87*	0.70
Corolla lobe length (mm)	7.0 (0.1)	6.7 (0.1)	3.05	0.80	0.76
Corolla diameter (mm)	15.3 (0.2)	15.2 (0.3)	0.22	3.08*	0.68
Corolla tube length (mm)	21.3 (0.5)	21.5 (0.6)	0.01	0.53	1.16
Calyx length (mm)	7.4 (0.1)	7.0 (0.1)	<u>8.31**</u>	<u>14.27***</u>	2.37
SLA ($cm^2 \ g^{-1}$)	93.5 (8.9)	73.7 (6.1)	<u>8.27**</u>	<u>6.54**</u>	<u>6.55**</u>
Photosynthesis ($\mu mol \ m^{-2} \ s^{-1}$)	2.80 (0.37)	1.66 (0.23)	<u>9.22**</u>	1.37	0.43
Stomatal conductance ($mol \ m^{-2} \ s^{-1}$)	0.21 (0.13)	0.06 (0.04)	<u>26.97***</u>	0.93	2.39
Integrative WUE ($\delta^{13}C$, ‰)	−37.34 (0.08)	−35.78 (0.24)	<u>38.93***</u>	<u>5.52*</u>	1.85

Treatment means are the average values (1SE) for plants grown in each of the two watering treatments, including all three population sources. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Underlined values were significant following the Holm's Bonferroni correction of P values

[a] Treatment df = 1, 84, except for SLA (df = 1, 82)

[b] Population df = 2, 84, SLA (df = 2, 82)

[c] Treatment × population df = 2, 82 for SLA
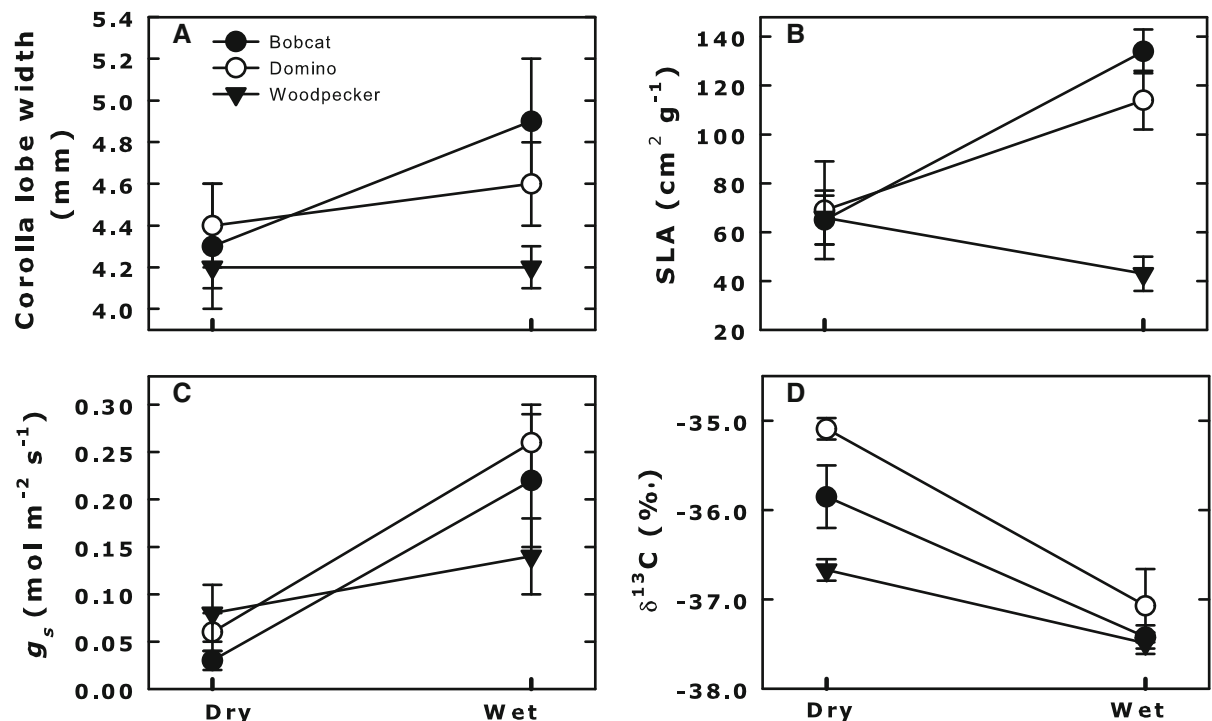
Fig. 3 Trait plasticity for *Leptosiphon androsaceus* in response to moisture availability in a growth chamber study. **a** Corolla lobe width (mm), **b** SLA ($cm^2 \ g^{-1}$), **c** stomatal conductance (g_s, mmol $H_2O \ m^{-2} \ s^{-1}$), and **d** integrated WUE ($\delta^{13}C$, ‰) were measured for plants grown from seeds originating from three different field populations under two different watering treatments. Traits shown are representative of floral, vegetative, and physiological traits in the study. See Table 4 for all measured traits and associated statistics

Table 4 Selection on trait means in response to water availability in *Leptosiphon androsaceus*

Trait	Well-watered β	Drought β
Corolla lobe width (mm)	0.14*	−0.13***
Corolla lobe length (mm)	0.24*	−0.07
Corolla diameter (mm)	0.06	−0.05
Corolla tube length (mm)	0.21*	−0.04
Calyx length (mm)	0.05	−0.06
SLA (cm^2 g^{-1})	0.04	−0.05
Photosynthesis (μmol m^{-2} s^{-1})	0.17*	0.01
Stomatal conductance (mol m^{-2} s^{-1})	0.12*	−0.04
Integrative WUE (δ^{13}C, ‰)	−0.06	0.01

Regression analysis was used to evaluate whether there was selection on traits within each treatment. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

experiment. A study such as ours, that includes both spatial and temporal variation in WUE, highlights how complex the responses to moisture availability may be.

The only trait that exhibited significant population differentiation in plasticity (*i.e.*, significant treatment × population interaction, Table 3) was SLA. Plants from the driest field population that exhibited the least inter-annual variation in moisture (Woodpecker) displayed the least plasticity in this trait, suggesting some variation in plasticity associated with drought across the precipitation gradient. Otherwise, all measured traits exhibited similar levels of plasticity across populations, regardless of variation in precipitation. This is consistent with observations of plasticity in *Phlox drummondii,* another member of the Polemoniaceae. Schlichting and Levin (1990) found similar levels of plasticity in response to moisture among seven populations that differed in precipitation. In spite of average differences among these *Phlox* populations in moisture, inter-annual variation in precipitation generated similar moisture heterogeneity across all populations over the long term.

Why does flower size vary across populations, if flower size is not plastic?

The pattern of decreasing flower size across populations with declining moisture availability is consistent with that observed in several studies (Herrera 2005; Lambrecht and Dawson 2007; Elle et al. 2010; Suárez et al. 2011; Lambrecht 2013). Our results stand in contrast, however, with those that show some variation in flower size is due to plasticity (Carroll et al. 2001; Elle and Hare 2002; Mal and Lovett-Doust 2005; Caruso 2006; Edwards et al. 2012). While corolla lobe

width and length and corolla diameter varied across populations in the field, we did not detect plasticity in the growth chamber study for any floral size trait other than calyx length. Our small sample size in the growth chamber study may have limited our ability to detect plasticity. This lack of observed plasticity occurred in spite of selection for larger flowers in the well-watered treatment and for smaller flowers in the drought treatment. These results support the variation observed in flower size in the field along the moisture gradient, in spite of pollinator preferences for larger flowers.

Constraints posed by rapid development associated with a drought escape strategy may contribute to small flower size in drier populations. In ephemeral or dry habitats, flower, leaf, and plant size are often small and WUE is low due to rapid development before the onset of drought (Elle et al. 2010; Franks 2011; Ivey and Carr 2012). Smaller flowers and plants and lower WUE in our drier populations are consistent with this strategy. However, plasticity toward increased WUE in drought and its independence from changes in flower size are not consistent with drought escape, suggesting that floral size variation in this species results from other factors.

A further possible mechanism for floral size variation and selection on floral size lies in floral water costs, and the constraints these pose on plant water balance and leaf physiology. Floral water costs have been shown to affect whole plant water balance and foliar photosynthesis (Galen et al. 1999; McDowell and Turner 2002). The magnitudes of these effects vary with moisture availability. For example, several studies, including one of the congeners *L. bicolor* measured over the same range as the current study (Lambrecht 2013), have documented a positive

correlation between flower size and WUE in dry locations (Lambrecht and Dawson 2007; Lambrecht 2013) and under dry conditions in greenhouse experiments (Kelly et al. 2008; Ivey and Carr 2012; Edwards et al. 2012), but the correlation disappears under well-watered conditions. These results suggest that foliar stomatal conductance is reduced, thereby increasing WUE, when water is limiting, perhaps to compensate for the loss of water from flowers. However, when water is not limiting, this foliar water control is not necessary. Moreover, in drier environments, plants may produce smaller flowers to reduce the surface area from which water may be lost (Galen et al. 1999; Lambrecht and Dawson 2007). In our drought study, fitness was positively correlated with smaller flowers in the dry environment, while larger flowers were selected in the wet treatment. Therefore, observed differences across our populations may reflect selection for small flowers in the drier populations as a mechanism to reduce water loss. Plasticity may also have contributed to these differences, even though we were unable to detect it. Perhaps, as a small plant with few leaves and a proportionally large corolla surface, floral water loss is disproportionately costly for *L. androsaceus*, as has been observed in other Mediterranean climate species (e.g., Teixido and Valladares 2014). These results have important implications for how this species responds to drought as California's climate changes, especially given the evidence that diversification within this genus may be tied to climate (Bell and Patterson 2000). While it is not unusual to consider how leaf drought-coping traits may evolve with climate change (e.g., Nicotra and Davidson 2010; Franks 2011), it is less common to consider how floral traits may respond.

Conclusions

Drought has presumably been a strong selective agent in plants. In this study, variation observed in *Leptosiphon androsaceus* across a gradient of moisture availability was due to a combination of selection and phenotypic plasticity in response to moisture availability. The patterns of spatial variation of WUE across populations *vs.* the direction of plasticity for that trait in response to drought are intriguing and demonstrate the complex response of this trait to moisture availability. Moreover, selection on flower

size suggests that floral water costs may contribute to the variation in floral size observed across populations varying in moisture availability. Further studies that consider how floral and other functional traits covary over space and time should prove insightful for understanding how drought produces variation in plant traits and provide information for determining how plants may respond to climate change.

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