Local adaptation mediates floral responses to water limitation in an annual wildflower

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PREMISE: Understanding how environmental stress affects the strength of mutualisms is critically important given observed and projected environmental changes. In particular, the frequency and duration of drought have been increasing worldwide. We investigated how water availability affects plant traits that mediate a pollination mutualism.

METHODS: For butterfly-pollinated Phlox drummondii, we determined how moisture availability affects flower size, nectar volume, and nectar sugar amount. Furthermore, we explored the role that local adaptation may play in responses to moisture availability by including plants collected from regions that differ in aridity. Finally, we determined whether responses of plant populations to selection may differ under drought by calculating heritability of traits under control and dry conditions.

RESULTS: Flower size was generally smaller in dry plants than in control plants. Early in the treatment period, nectar volume and sugar were higher in dry plants than in control plants for plants from both arid and wetter regions. With prolonged dry treatment, nectar volume and sugar remained higher only in plants from the arid region. Heritability of floral traits was lower for water-limited plants than for control plants.

CONCLUSIONS: Plant investment into pollination mutualisms under environmental stress may depend on the extent to which populations are already locally adapted to such conditions, suggesting that mutualism may remain strong, at least in arid regions. However, decreases in heritability under water-limitation suggest that responses to selection imposed by pollinators may be low, even if drought-adapted plants maintain production of rewards to pollinators.

KEY WORDS   drought; floral display; flower size; heritability; local adaptation; mutualism; nectar; phenotypic plasticity phlox; pollinator; pollinator-mediated selection.

The frequency and duration of extreme environmental events is increasing worldwide (Ummenhofer and Meehl, 2017). Understanding the way that environmental changes affect the eco-evolutionary dynamics of cooperative interactions among species, or mutualisms, is thus important. In particular, there is growing concern that environmental change will negatively affect plant–pollinator mutualisms, which are key to both ecological community health and human food and resource availability. Here we investigated response to water limitation in plant traits that both attract and reward pollinators.

Species can withstand environmental stress via individual phenotypic plasticity, population local adaptation, or both. Phenotypic plasticity (hereafter plasticity) is the manifestation of different phenotypes from the same genotype in different environments (Fordyce, 2006). Plastic responses to environmental stress can increase or decrease the strength of a mutualism depending on the direction of

the response in traits that mediate the mutualism (hereafter mutualism traits). Mutualism requires that benefits are exchanged between partners, and thus, mutualisms weaken if organisms decrease the amount of reward exchanged (Kiers et al., 2010). If there is genetic variation for mutualism traits in a population, phenotypic plasticity could also weaken mutualism by shielding certain genotypes from selection (Hegland et al., 2009). However, if there is genetic variation for plasticity ($G \times E$) such that the magnitude or direction of plastic responses differs among genotypes, selection could favor genotypes that maintain or even increase investment in mutualism traits under environmental stress. Genetic variation for plasticity may be expected in species that exist along environmental gradients, with stress-adapted populations being better able to maintain investment in mutualism under challenging environmental conditions.

Variation in mutualism traits across space and time in response to environmental stress can destabilize mutualisms if this variation alters the communication between partners. Individuals of many species choose their mutualistic partners by assessing traits of potential partners that are associated with partner quality (Schaefer et al., 2004; Razo-Belman et al., 2018). Traits of individuals of one species that can be perceived by individuals of another species, and that are associated with partner quality, are known as honest signals. Honest signals contribute to the evolutionary stability of mutualisms because cooperative individuals often have the opportunity to interact with multiple partners of differing quality (Noë and Hammerstein, 1995; Bronstein, 2001), and honest signals allow individuals to direct benefits to other cooperative individuals (Edwards and Douglas, 2007). When resources are scarce, trade-offs in resource allocation may become more pronounced and signal–reward relationships could change due to lower investment in signal or reward.

Plant–pollinator interactions are classic examples of how signals and rewards influence mutualisms (Waddington and Holden, 1979) and how environmental change affects mutualism (Memmot et al., 2007; Schweiger et al., 2010). Pollinators use a variety of floral traits to locate plants and assess reward quality (Schaefer et al., 2004). Pollinators can learn to associate various morphological or chemical characteristics of flowers with reward (Meléndez-Ackerman et al., 1997; Weiss, 1997). They will often continue to forage on flowers with the same characteristics of previously rewarding flowers (Waser, 1986), which facilitates the reproductive success of plants. The maintenance of signal–reward associations in plants is therefore straightforward if the signal itself is the reward, for example, when nectar or pollen emit visual or olfactory signals to pollinators (Howell and Alarcón, 2007). In cases in which the signal itself is not the reward, for example, when visual floral characteristics are used as signals (Schaefer et al., 2004), signal–reward associations can be maintained by genetic constraints (Smith, 2016), or by selection for avoidance of cheating plants by pollinators (Benitez-Vieyra et al., 2014). Mutualism could thus be weakened if the correlation between floral reward and traits that pollinators use to assess reward quality decreases under environmental stress.

Drought may be a particularly important destabilization mechanism for plant–pollinator mutualisms because plants often exhibit phenotypic variation in traits that are important both in attracting and rewarding pollinators under varying water availability. Pollinators often prefer plants with larger floral displays (number of flowers), or flower size (signals to pollinators; Eckhart, 1991; Thompson, 2001; Goulson, 2010; Sletvold et al., 2017). However, decreased water availability is often associated with smaller floral display or flower size (Carroll et al., 2001; Opedal et al., 2016; Gallagher and Campbell, 2017; Descamps et al., 2018; Phillips et al., 2018). Decreased water availability is also often associated with lower volumes of nectar produced (a pollinator reward; Carroll et al., 2001; Halpern et al., 2010; Waser and Price, 2016; Gallagher and Campbell, 2017; Descamps et al., 2018). In greenhouse settings, Waser and Price (2016) found a positive association of soil moisture and nectar sugar for *Ipomopsis aggregata* (scarlet gilia, Polemoniaceae). Similarly, Descamps et al. (2018) found a decrease in nectar sugar under increasing water stress, but Carroll et al. (2001) found no effect of watering treatment on nectar sugar for *Epilobium angustifolium* (fireweed, Onagraceae). The reason for this discrepancy is unknown and motivates further investigation. In particular, variation in response to water availability may reflect differences in local adaptation to drought of the populations studied or differential plasticity in trait expression.

We used the butterfly-pollinated annual plant *Phlox drummondii* (Polemoniaceae) to explore how water availability affects plant floral traits and the role that local adaption plays in drought response. Butterflies use visual cues presented by *P. drummondii* to choose plants on which to forage (Hopkins and Rausher, 2012, 2014; Briggs et al., 2018). Using controlled growing conditions, we experimentally simulated water limitation for *Phlox drummondii* plants that were grown from seeds collected along a strong moisture gradient in central Texas. We asked (1) how does water limitation affect floral display, nectar volume, and nectar sugar content; (2) how does the effect of water limitation on traits vary among plants from different native moisture regimes; and (3) what are phenotypic and genetic correlations among traits, and how do these differ between water-limited and control conditions. Furthermore, we calculated heritability of traits under control and dry conditions to determine responsiveness of these traits to potential pollinator mediated selection under drought.

MATERIALS AND METHODS

Study system

Phlox drummondii is an annual herb native to Texas that is often found in meadows, along roadsides, in pastures, and in other disturbed areas. Seeds germinate in late fall or early spring, and plants flower and set fruit during the spring and summer. Most *P. drummondii* are self-incompatible (Erbe and Turner, 1962; Levin, 1978; Roda and Hopkins, 2018). The primary pollinator species are lepidopterans, including *Battus philenor*, and several skipper species (Hopkins and Rausher, 2012, 2014). *Phlox drummondii* grows across a moisture gradient from relatively wet east-central Texas to relatively dry west-central Texas.

Common garden experiment

During the spring of 2015, we collected open-pollinated seeds from plants in seven populations across central Texas (Appendix S1). We brought seeds back to Harvard University's Arnold Arboretum and allowed them to ripen. We planted 8–20 seeds per maternal plant, with the aim of using eight half siblings per maternal plant. Germination was variable, and our final sample included 139 plants from 23 maternal half-sibling families, 1–8 plants per maternal family (average 6), and 1–4 maternal families per population (average 3.3; Appendix S1).

We cold-stratified seeds for 1 week at 4°C in a cold chamber, and then planted them in Fafard super-fine germination soil (Sungro Horticulture, Agawam, MA, USA) and allowed them to germinate in growth chambers (Conviron MTPC144, Winnipeg, Manitoba, Canada) set to long days (16 h of light) and kept at 27°C. Seedlings were then transferred to preweighed pots containing Promix (Quakertown, PA, USA) high porosity soil with mycorrhizae. The initial dry pot mass was used to calculate belowground biomass at the end of the experiment (see below). We watered plants as needed and fertilized them regularly with Dyna-Gro Grow (Richmond, CA, USA) prior to blooming and Dyna-Gro Bloom after initiation of flowering. We did not fertilize plants after initiation of the water treatment.

Imposition of water stress

We initiated the water treatment after all plants had begun flowering. This treatment schedule mimics the transition from spring rains to dry summer that is characteristic of the natural growing season of *P. drummondii*. We haphazardly assigned half the individuals from each half-sibling family to either the control (well-watered) or dry treatment (which, for simplicity we will refer to as "dry"). We fully saturated all control and dry treatment pots with water and measured pretreatment saturated mass. We monitored plants daily as they underwent a controlled dry-down to a target treatment mass that was reflective of percentage soil saturation. We allowed control plants to dry to 50% soil saturation and dry plants to 10% soil saturation. At 10% soil saturation, plants began to wilt slightly. We weighed pots daily to ensure that all plants in a treatment were within 10–15% soil moisture of each other and added water to pots that dried too rapidly. All plants reached their target soil moisture concentration 20 d after the targeted dry-down began, and we maintained dry and control pots at their target soil saturation for 12 d before rewatering. See Appendix S2 for details of how consistent soil saturation was maintained.

We evaluated how watering treatments affected water status in two ways. First, we assessed the physiological outcomes of the two treatments by measuring leaf relative water content. We selected one leaf from each plant that was second from the base of a lateral stem. We then weighed and submerged each leaf cut side down into a 1.5 mL centrifuge tube full of deionized water. Tubes were placed in the refrigerator for 14 h after which leaves were again weighed. Leaves were then placed in a drying oven for 24 h, and weighed a third time. Leaf relative water content was calculated as (fresh mass – dry mass)/(saturated mass – dry mass). We compared leaf relative water content between dry and control plants using a linear mixed model with treatment as the dependent variable and maternal family as a random effect and implemented the model using the R package lme4 (Bates et al., 2015a, b).

Second, we assessed leaf water potential for one dry plant and one control plant from 13 maternal families using a pressure bomb. We used on average 0.23 g (range: 0.1–0.43 g) of the top of a stem, clipping from where that stem was 1 mm wide. We measured water potential 2 d after removing flowers during our second set of trait measurements (early treatment; see below) so we chose only stems that had no flowers or buds.

Trait measurements

We measured plant traits at four time points throughout the experiment: before the dry-down began (pretreatment, PT), 7 d into the treatment when plants reached their target soil saturation (early treatment, ET), 12 d into the treatment (late treatment, LT), and 7 d after rewatering (RW). At each time point, we measured the following floral traits on one haphazardly chosen flower per plant: petal length, corolla length, nectar volume, nectar sugar (sucrose) content. Petal length and corolla length were measured using digital calipers. Nectar volume was measured using microcapillary tubes, and percentage sucrose was measured using a refractometer. The amount (mg) of sucrose per flower was assessed using the method of Bolten et al. (1979). To control for flower age, we removed all flowers from plants 3 d before trait measurement, making all measured flowers between 1 and 3 days old. We also measured the number of flowers produced 3 d before ET and LT trait assessments, daily water use during the treatment period, and above- and belowground biomass following the experiment. Appendix S3 shows a flow chart of our experimental procedures.

Characterization of native moisture regimes

We extracted data from the WorldClim database (v1.4) corresponding to each population's geographic coordinates using the R packages raster (Hijmans et al., 2016), maptools (Bivand and Lewin-Koh, 2015), sp (Pebesma and Bivand, 2005), and rgdal (Bivand et al., 2016). We incorporated the 19 available bioclimatic variables with a 30-s resolution into a principal component analysis (PCA) implemented in R using the factoextra package (Kassambara and Mundt, 2017). Variables associated with rainfall contributed to the first principal component, which was associated with longitude, and explained 51.2% of the variation (Appendices S4, S5). For example, the average precipitation in the driest month was 47.5 mm for the eastern populations and 30 mm for the western populations (Appendix S4). The seven populations formed two distinct clusters along this principal component (Appendix S6), with the three western populations making up one group and the four eastern populations making up the other group. We therefore divided the populations into western and eastern groups and assessed traits for each group.

To explore whether populations are differentially adapted to native moisture regimes we evaluated whether plants from the wetter and drier regions differed in the fraction of biomass invested in roots, which is a phenotype that is associated with drought adaptation (Poorter et al., 2012). To calculate the root to shoot ratio we measured dry above- and belowground biomass of each plant at the end of the experiment. After rewatering plants, we clipped each plant just above the soil, weighed each plant, allowed plants to dry completely, and reweighed each plant. To calculate belowground biomass we allowed pots to dry completely, and subtracted initial dry pot mass from dry pot mass at the end of the experiment (with roots). We also calculated average daily plant water use for the period during which all plants were at their target saturation (50% for control plants or 10% for dry plants) as the average daily mass difference between pots on day t (after watering to target soil percentage) and day $t + 1$ (before watering).

Modeling trait variation

We determined how traits differed between treatments and regions at each time point using linear mixed models (LMM) implemented with the lme4 package in R (Bates et al., 2015a, b). The trait of interest was the dependent variable, treatment (dry

or control) and region were fixed factors, and maternal family was a random factor. We also included the order in which plants were measured as a continuous variable to account for location in the growth chamber and the fact that nectar accumulates within flowers over a day.

For the floral traits (nectar volume, sucrose content, petal length, and corolla length), we ran linear mixed models for each trait for each time point. We log-transformed the nectar traits, for which initial model residuals were not normally distributed (nectar volume and sucrose amount). We determined whether treatment and region affected the number of flowers produced at the ET and LT timepoints, and how treatment and region affected final aboveground and belowground dry biomass after rewatering. We also modeled water use over the entire treatment period. We used daily water use as the dependent variable, region, treatment, and day as fixed factors, and maternal family as a random effect. We evaluated whether fixed factors and their interactions significantly improved the model by sequentially dropping each term from the model and conducting likelihood ratio tests on the nested models, using the lmtest package (Zeileis and Hothorn, 2002). We considered a significant interaction term between treatment and region evidence that there was genetic variation for plasticity (G × E). We evaluated whether maternal family improved the model using the Akaike information criterion (Sakamoto et al., 1986). If including an interaction significantly improved the model, we used post hoc tests to compare least square means (lsmeans) using the emmeans function from the multcomp package (Hothorn et al., 2008).

We note that although we took repeated measures from the same plants over time in this study, the dynamics of our treatments make it logical to analyze each time point separately for the floral traits. Rather than exploring how traits change over time, we were interested in exploring differences between regions and treatments in responses to water limitation, and differences between regions and treatments in their recovery after rewatering. In addition, plants in the water-limited and control groups experienced the same conditions pretreatment and at rewatering (the first and last time points), which makes interpretation of a single model that incorporates all time points complicated. Of note, if we included time as a factor in the models, there was a significant time × treatment × region three-way interaction for all the traits that were measured at all time points, except corolla length. This three-way interaction justifies further investigation of the treatment and region effects at each time point. We choose to not include the four time points in one model because the interpretation of such a complicated interaction term is not intuitive. We therefore present results from separate models containing data from each time point.

Trait correlations and heritability

We estimated phenotypic correlations using the cor.test function implemented in R and estimated the overall statistical significance of multiple comparisons within each group using the Benjamini and Hochberg (1995) method, with a false discovery rate of 0.05. This method controls the level of falsely rejected null hypotheses while correcting for an increase in type I error (Benjamini and Yekutieli, 2001; Narum, 2006). We estimated genetic covariance and correlations using multivariate models implemented using asreml (VSNI, Hemel Hempstead, UK). To avoid scaling issues, we first

transformed trait values so that they had unit variance. We then estimated the statistical significance of each covariance component by comparing the log likelihood of the full model to that of a model in which the covariance was constrained to zero.

To estimate heritability (h^2) of traits, we partitioned total phenotypic variance into additive genetic variance (V_A) and residual variance (V_R) using LMMs with the same fixed and random effects as described above, and we implemented these using the R package asreml-R v.3.0 (Butler et al., 2009). We then estimated heritability as $h^2 = V_A/(V_A + V_R)$, where V_A is the variance explained by maternal half-sibling family and V_R is the residual variance from the LMM. To calculate confidence intervals of h^2, we used the pin function from R package nadiv (Wolak, 2012). To gain insight into whether there was significant additive genetic variance, we used likelihood ratio tests to compare models with and without the random effect of maternal half-sibling family.

RESULTS

Effectiveness of water treatments

The dry treatment significantly decreased leaf relative water content relative to the control treatment (mean LRW content: 0.81 g for dry plants, 0.89 g for control plants; LMM est. = 0.08; $P = 0.02$). Similarly, water potential was significantly lower for control plants (t-test, $t = 5.0$, df = 23, $P < 0.001$).

Differences among regions and treatment groups

Our data suggest that eastern and western plants are differentially adapted to their respective moisture regimes. Control plants from the western, drier region had a higher root to shoot ratio than plants from the wetter, eastern region (see Table 1 for means and model outputs). In the control plants, we found variation in nectar through time between regions. Specifically, young control plants (our early measurements) from the drier western region produced more nectar, with a higher sugar content, than eastern plants did (Table 1). This pattern was reversed as plants aged, with the eastern plants producing more nectar, with a higher sugar content, than western plants (Table 1). In general, control plants from the eastern region produced flowers with longer petals and corollas throughout the experiment than did control plants from the western region (Table 1).

There was phenotypic plasticity in response to moisture availability for all traits, and there was genetic variation for plasticity for several traits such that responses to water limitation varied according to native moisture regime (Fig. 1, Table 1). Surprisingly, both nectar volume and sucrose content were higher for dry plants than control plants at the early-treatment measurement (ET) for plants from both regions. Nectar volume and sucrose amount remained higher for western plants in the dry versus control treatment at the late-treatment measurement (LT) (Fig. 1, Table 1). In contrast, eastern plants produced less nectar and sucrose in the dry treatment than control treatment at LT (Fig. 1, Table 1).

Western and eastern plants also responded differently to treatment in terms of the number of flowers produced, the root to shoot ratio, and water use. Dry treatment plants from both regions produced fewer flowers, but the difference in flower number between control and dry plants was greater for western plants (Table 1, Fig. 1). Western control plants produced less aboveground biomass

TABLE 1. For each time point (Time), trait means, and standard errors for eastern and western plants pretreatment (PT), during early treatment (ET), during late treatment (LT), and after rewatering (RW), for eastern control and dry (EC & ED), western control and dry (WC & WD) plants, and interaction estimate (Int. est.), treatment, or region estimate if significant, as assessed by comparing the log likelihood between nested linear mixed models with and without the interaction or factor of interest. *P*-values are in parentheses. Traits assessed from top to bottom include nectar volume ("N"), sucrose amount ("Sucrose"), petal and corolla length, number of flowers, fresh aboveground biomass ("Mass"), water use ("Water"), and root to shoot mass ratio ("R:S").

Trait	Time	Mean EC	Mean ED	Mean WC	Mean WD	Int. est.	Region est.	Treatment est.
N (µL)	PT	0.032 ± 0.006		0.038 ± 0.005		NA	0.0049 (0.007)	NA
	ET	0.1 ± 0.02	0.14 ± 0.03	0.16 ± 0.029	0.19 ± 0.044	−0.0047 (0.037)	0.042	−0.028
	LT	0.16 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.15 ± 0.04	ns	−0.011 (0.019)	−0.013 (<0.001)
	RW	0.25 ± 0.04	0.21 ± 0.03	0.21 ± 0.04	0.32 ± 0.05	ns	0.007 (0.028)	0.018 (0.025)
Sucrose (mg)	PT	0.011 ± 0.002		0.016 ± 0.0029		NA	0.005 (0.006)	NA
	ET	0.041 ± 0.007	0.046 ± 0.01	0.059 ± 0.01	0.063 ± 0.013	0.0015 (0.014)	0.017	0.002
	LT	0.079 ± 0.01	0.056 ± 0.009	0.047 ± 0.009	0.053 ± 0.01	0.029 (0.045)	−0.03	−0.02
	RW	0.12 ± 0.02	0.093 ± 0.01	0.081 ± 0.01	0.12 ± 0.02	ns	−0.01 (0.013)	−0.0031 (0.0088)
Petal (mm)	PT	12.5 ± 0.11		11.3 ± 0.16		NA	−1.2 (<0.001)	NA
	ET	12.1 ± 0.23	11.5 ± 0.27	11.8 ± 0.24	11.4 ± 0.28	ns	ns	−0.54 (0.0074)
	LT	12.9 ± 0.15	12.4 ± 0.40	12.5 ± 0.28	11.7 ± 0.29	ns	−0.55 (0.04)	−0.71 (0.011)
	RW	13.3 ± 0.23	13.3 ± 0.27	12.8 ± 0.31	13 ± 0.27	ns	−0.52 (0.041)	ns
Corolla (mm)	PT	16.3 ± 0.17		15.3 ± 0.24		NA	−0.98 (0.001)	NA
	ET	16.7 ± 0.27	16.5 ± 0.33	15.6 ± 0.27	15.2 ± 0.26	ns	0.01 (<0.001)	ns
	LT	16.7 ± 0.24	16 ± 0.0.40	15.9 ± 0.30	14.9 ± 0.26	ns	−0.88 (0.0036)	−0.92 (0.0021)
	RW	16.9 ± 0.31	16.4 ± 0.29	16.1 ± 0.30	15.7 ± 0.31	ns	−0.77 (0.011)	ns
Flowers	ET	15 ± 1.6	11 ± 1.5	16 ± 2.5	10 ± 1.4	−3.1 (<0.001)	0.72	−3.7
Flowers	LT	21 ± 1.9	12 ± 1.6	24 ± 3.1	11 ± 1.4	−3.7 (<0.001)	2.2	−8.7
Mass (g)	RW	27 ± 1.3	23 ± 1.3	23 ± 1.7	18 ± 1.1	ns	−4.3 (0.0058)	−4.5 (<0.001)
Water (mL/day)	RW	50 ± 2.9	28 ± 1.4	51 ± 3.8	28 ± 1.8	−1.8 (0.02)	0.89	−21
R:S	RW	14 ± 1.2	18 ± 1.3	26 ± 3.1	27 ± 2.2	−0.76 (0.038)	11.5	3.1

and had a greater root to shoot ratio than eastern control plants (Table 1, Fig. 1). Plants from both regions in the dry treatment had a higher root to shoot ratio than control plants. The difference between control and dry treatment plants was greater for eastern plants, but eastern plants had far lower root to shoot ratios than those for western plants across both treatments (Table 1, Fig. 1). Dry plants from both regions had lower water use than control plants, and the difference in water use between control and dry plants was greater for western plants (Table 1; Appendix S7).

Phenotypic correlations

Table 2 shows correlations among floral traits. Nectar volume and nectar sugar were positively correlated in all groups (eastern and western dry and control groups), and corolla length and petal length were positively correlated in all groups except eastern dry plants. Interestingly, western dry plants comprised the only group for which there were significant correlations between signal and reward traits. In these plants, we found a positive correlation between sucrose amount and petal length and a negative correlation between the number of flowers and nectar volume.

Heritability and genetic correlations

Point estimates of heritability of most traits varied across treatments. Nectar volume, petal length, number of flowers, and water use had lower heritability in the dry treatment then in the control treatment (Fig. 2; Appendix S8). The pattern for nectar sugar was more complex, and heritability of corolla length seemed to increase with plant age for both dry and control groups (Fig. 2). Point estimates of heritability of final dry biomass and the root to shoot ratio were higher for dry plants then control plants (Appendix S8). All standard error bars overlapped with zero for non-zero measures of heritability (Appendix S8). There

were no consistent patterns for whether additive genetic variance was significant across traits, time points, and treatments (Appendix S8). Sample sizes hindered our ability to detect significant heritability and genetic correlations among traits, but there was one significant genetic correlation between petal length and corolla length, for the dry plants at the first time point (Appendix S9).

DISCUSSION

We examined how drought may affect plant–pollinator mutualisms by determining how water limitation affects the quality of reward to pollinators, floral display, signal–reward relationships, and heritability of floral traits. The way that populations can respond to drought may depend on the extent to which they are adapted to their current environment (Schlichting and Pigliucci, 1998). We characterized responses of plant traits to water limitation across the east–west moisture gradient of the *P. drummondii* range. In general, we found an increase in nectar and sugar production per flower in response to limited water availability. This response varied across the range of *P. drummondii*, with plants from drier areas showing more of an increase in floral reward due to water limitation. We found a correlation between reward traits and signal traits only for the moisture-limited plants from the dry region. Finally, we found a general decrease in heritability of reward and signal traits in dry environments suggesting decreased effectiveness of selection in generating evolutionary responses in plant populations under increasing drought.

In many plant–pollinator mutualisms, the quality of the food reward determines whether pollinators continue to visit plants of a given species (Waser, 1986). The pollinators of *P. drummondii* are nectivorous butterflies, for which the quality of the food reward depends on the amount of nectar produced, as well as the total amount of sugar present in the nectar (Kim et al., 2011). In other plant species, nectar volume responds plastically to water availability, such that increasing

water is associated with higher nectar volumes (Wyatt et al., 1992), and decreased water availability is associated with lower volumes (Carroll et al., 2001; Halpern et al., 2010; Waser and Price, 2016). In addition, the amount of sugar in nectar is either positively associated with moisture availability (Waser and Price, 2016) or not associated (Carroll et al., 2001). Surprisingly, our experimental water limitation revealed that dry plants produced more nectar and nectar with a higher sugar content than in well-watered plants. This is interesting given that nectar is predominantly composed of water and sugar, both of which are likely limited in dry conditions. Furthermore, photosynthetic carbon assimilation can drop due to stomatal closure (Lawlor and Cornic, 2002) and decrease the availability of nonstructural carbohydrates such as sucrose (Maguire and Kobe, 2015).

What explains the difference between the results we have documented for *P. drummondii* and previous studies on how water availability affects nectar quality? We outline two possibilities. First, phenotypic responses of plants to drought often differ along environmental gradients (Carvajal et al., 2017; Sánchez-Salguero et al., 2018). However, most studies that examined how nectar characteristics relate to moisture level focused on populations from only one geographic area (Teuber and Barnes, 1979; Carroll et al., 2001; Halpern et al., 2010; Waser and Price, 2016). Our inclusion of plants from different native moisture regimes allowed us to detect within species variation in responses to water availability. We found marked differences between western and eastern plants in how nectar volume and sugar amount varied between dry and well-watered plants. These differences were especially evident for late treatment measurements, when western dry treatment plants
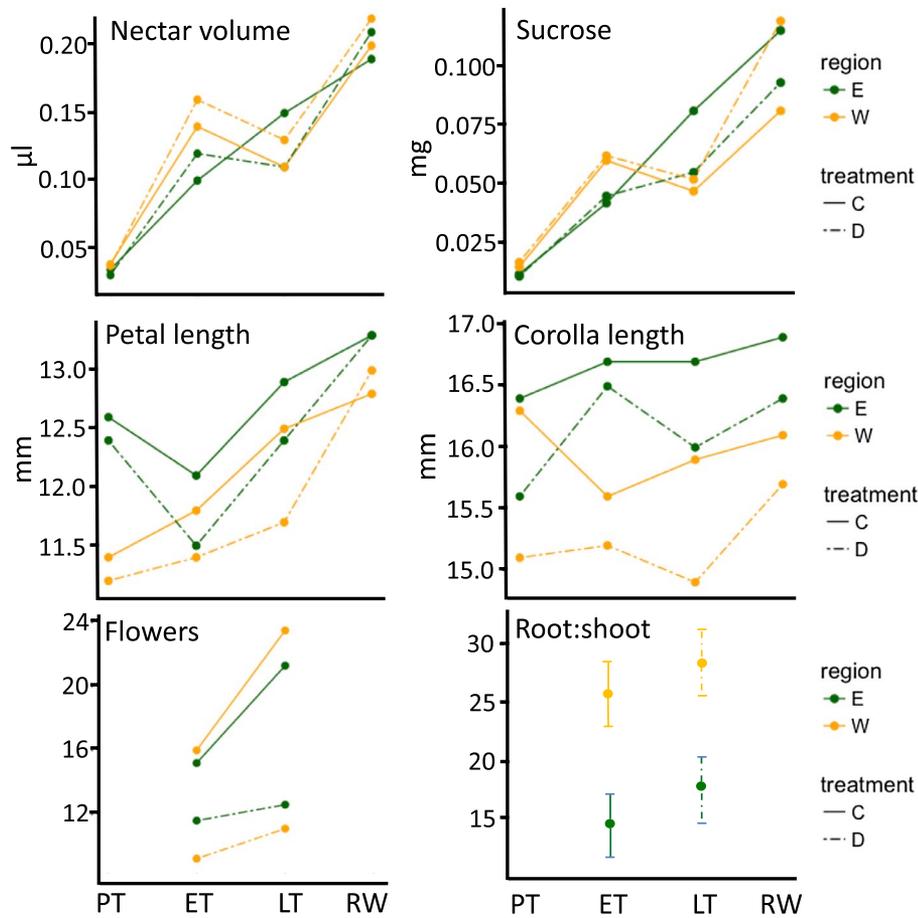

FIGURE 1. Least square means for six traits at four time points: pretreatment (PT), early treatment (ET), late treatment (LT), and after rewatering (RW). Solid lines represent control plants (C), dashed lines represent dry plants (D), orange represents western plants (W), and green represents eastern plants (E). See Table 1 for actual means and results of models that assess differences among regions and treatments within time points.

TABLE 2. Phenotypic correlations for traits during late treatment, including eastern control (EC; top table, top right, above dashes), eastern dry (ED; top table, bottom left, below dashes), western control (WC; bottom table, top right, above dashes), and western dry (WD; bottom table, bottom left, below dashes) plants. Traits assessed included nectar volume, nectar sucrose amount, petal length, corolla length, and number of flowers produced. *P*-values calculated before adjusting for multiple comparisons are in parentheses; *P*-values that remained significant after adjusting are in bold.

|	EC				
	Nectar vol.	**Sucrose**	**Petal**	**Corolla**	**Flowers**
ED					
Nectar vol.	—	0.87 (<0.001)	0.26 (0.11)	0.28 (0.08)	0.14 (0.41)
Sucrose	0.75 (<0.001)	—	0.24 (0.13)	0.35 (0.027)	0.16 (0.31)
Petal	0.19 (0.36)	0.36 (0.07)	—	0.4 (0.01)	−0.24 (0.13)
Corolla	0.18 (0.39)	−0.05 (0.80)	−0.08 (0.70)	—	−0.08 (0.64)
Flowers	0.08 (0.70)	−0.07 (0.75)	−0.47 (0.016)	−0.04 (0.85)	—
	WC				
	Nectar vol.	**Sucrose**	**Petal**	**Corolla**	**Flowers**
WD					
Nectar vol.	—	0.92 (<0.001)	−0.015 (0.93)	0.15 (0.41)	−0.21 (0.24)
Sucrose	0.93 (<0.001)	—	0.09 (0.60)	0.25 (0.16)	−0.11 (0.53)
Petal	0.32 (0.08)	0.47 (0.009)	—	0.66 (<0.001)	0.12 (0.50)
Corolla	−0.05 (0.80)	−0.02 (0.93)	0.55 (0.0015)	—	0.35 (0.048)
Flowers	−0.45 (0.01)	−0.36 (0.05)	0.15 (0.42)	0.40 (0.03)	—
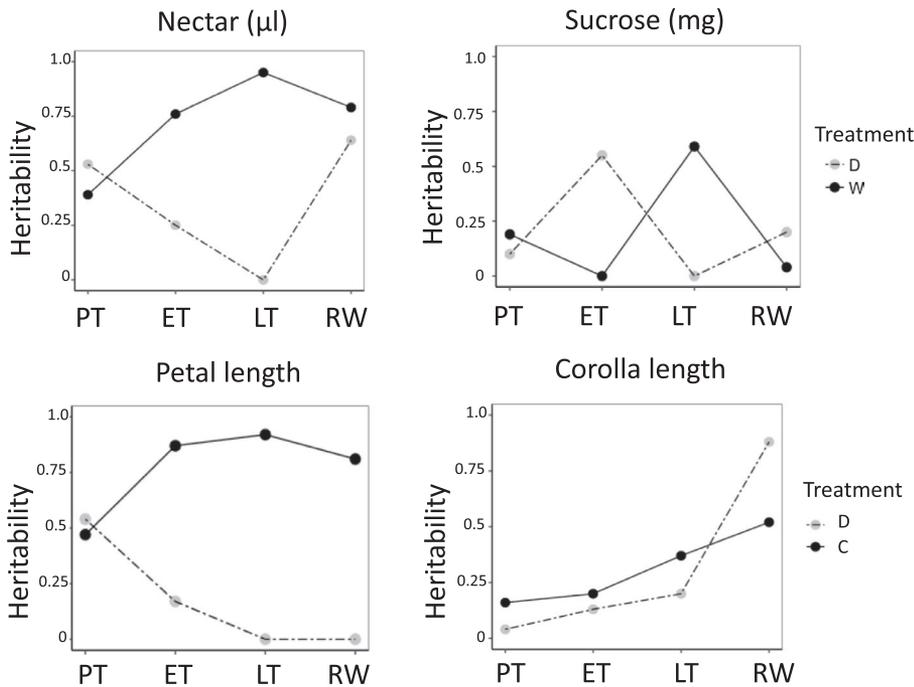
FIGURE 2. Heritability estimates of traits at four time points: pretreatment (PT), early treatment (ET), late treatment (LT), and after rewatering (RW). Gray dashed lines represent dry plants, and solid black lines represent control plants. See Appendix S8 for standard errors.

produced more nectar with higher sugar content than well-watered plants, but the pattern was the opposite for eastern plants, for which dry plants produced less nectar with lower sugar content (Table 1, Fig. 1). This result suggests that eastern and western plants have different resource allocation strategies in terms of investment in nectar quality versus other traits. Indeed, both eastern and western dry plants had smaller flowers and fewer flowers than well-watered plants, yet the difference between watering treatments in the number of flowers and flower size was greater for western plants than it was for eastern plants (Appendix S7).

Second, our experimental design, in which we examined trait changes over time, may have allowed us to detect nuances of drought response undetectable from a single point in time. We examined how nectar traits differed between dry and well-watered plants twice after plants reached their target soil saturation percentages. The relationship between average nectar volume and sugar content of dry and well-watered plants changed between measurement times for plants from the wetter, eastern populations. Had we quantified nectar volume and sugar content only during late treatment, and only for plants from the wetter eastern population, our pattern would have been similar to that of the previous studies, and we would have concluded that water limitation decreases nectar quality, although the true response is clearly more complex.

Unlike nectar quality, responses of floral display to water limitation were largely consistent across regions and time points, with both regions showing decreased floral size for dry plants relative to well-watered plants. However, plants from the western, drier region showed greater floral trait plasticity in response to water limitation than plants from the east. The difference in plasticity between eastern and western regions was most evident for the number of flowers produced. Dry plants from both regions produced fewer flowers than well-watered plants, but the difference between treatments was greater for western plants. What explains the difference in plasticity between regions? Populations can adapt to novel environmental conditions via the non-mutually exclusive mechanisms of phenotypic plasticity or ecotypic variation (Valladares et al., 2014). Populations that have experienced greater heterogeneity in soil moisture often show higher levels of phenotypic plasticity in functional traits (Gianoli and González-Teuber, 2005; Lázaro-Nogal et al., 2015; Carvajal et al., 2017). The precipitation seasonality, estimated as the coefficient of variation (from www.worldclim.org), is significantly higher (t-test, 6 df, $P = 0.03$) for western populations (average 34.75) than eastern populations (average 30.67). The greater plasticity we see in western plants is consistent with these populations evolving greater plasticity in response to greater variability in environmental conditions.

The greater plasticity of western plants was also evident in how signal–reward associations changed under water limitation. We hypothesized that trade-offs in resource allocation would become more pronounced under drought and signal-reward relationships would change due to lower investment in signal or reward. Interestingly, there was no association of any aspect of floral display that we measured and nectar quality for most plants. Only for the western dry plants did we find a significant and positive correlation of petal size and nectar sugar amount (Table 2) and a negative correlation between flower number and nectar volume. The positive correlation between petal size and nectar sugar indicates that petal size could be used by pollinators as an indicator of partner quality, even under water-limited conditions. The maintenance of energetic reward to pollinators is likely driven by a trade-off between investment in nectar quality and number of flowers produced.

The response to limited water availability in floral display and reward suggests the mutualism between *P. drummondii* and its butterfly pollinators could be strengthened under drought. *Phlox drummondii* often grows in patches of many individual plants. If pollinators use floral display of many co-flowering plants to locate a place to forage for food, but then once within the patch, use flower size to choose among flowers to visit, the honest signal of larger petals having greater rewards could maintain floral fidelity of pollinators to *P. drummondii* under stressful environmental conditions. If, however, pollinators choose among plants based on the number of flowers per plant, the dishonesty of this signal under drought might deter pollinators from continuing to forage on *P. drummondii*. While there has been no work investigating the role that flower size versus flower number may play in plant choice by pollinators in our system, butterfly pollinators of *P. drummondii* do discriminate among *P. drummondii* plants based on other floral traits such as flower color (Hopkins and Rausher, 2014; Briggs et al., 2018). Consistency in signal–reward associations promotes pollinator fidelity in other systems (Benitez-Vieyra et al., 2010), suggesting the need for future research

investigating butterfly preference for flower size and flower number for *P. drummondii*.

Our study focused on plant response to highly controlled growth conditions that would be largely infeasible to maintain in the field with natural pollinator selection. But, our findings motivate future work in this system investigating pollinator behavior toward plants that have experienced moisture limitation. Specifically, finding that pollinators prefer plants from dry areas over wet areas under water-limited conditions would indicate that the drought response of plants from western populations is adaptive and could have resulted from past pollinator-mediated selection. Selection by pollinators on floral traits is common in nature (reviewed by Parachnowitsch and Kessler, 2010; Sletvold and Ågren, 2010; Ågren et al., 2013; Schiestl and Johnson, 2013; Trunschke et al., 2019), as is selection by other agents (Strauss and Whittall, 2006; Ågren et al., 2013). In an experimental field study, Gallagher and Campbell (2017) found that non-drought-treated plants had larger corollas and received more pollinator visits than drought-treated plants, which translated to a difference in seed set. Gallagher and Campbell did not assess heritability of corolla shape, so the potential response of this trait to selection by pollinators is unknown. In our study we found genetic variation for plasticity, but none of our estimates of heritability are significant. The lack of significance is likely due to sample sizes being too small to detect a significant effect of maternal family on trait variation. Nevertheless, a qualitative examination of our results suggests that heritability of nectar volume and petal size decreases under water-limitation. Thus, future work that assesses pollinator-mediated selection on floral traits for moisture-limited and control plants from across the environmental moisture gradient will clarify whether phenotypic responses of western plants reflect an adaptation to maintain pollinator visitation under moisture-limited conditions and will provide insight into how populations may evolve under future environmental stress.

CONCLUSIONS

Our results suggest that the way populations respond to drought depends on the extent to which they are adapted to their environment (Schlichting and Pigliucci, 1998). Drought-adapted plants may be more likely to show phenotypes that will facilitate successful pollination during periods of water limitation, including the maintenance of high-quality nectar reward, and an association of other floral traits with this reward. However, reduced heritability of floral traits may result in lower responses of plant populations to pollinator-mediated selection under drought.

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AUTHOR CONTRIBUTIONS

S.S., B.A., and R.H. designed the experiment. S.S. and B.A. conducted the experiment, and S.S. performed the data analysis and wrote the manuscript with input from R.H.

DATA AVAILABILITY

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5td7f6m (Suni et al., 2019).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Site locations and sample sizes.

APPENDIX S2. Maintenance of consistent percentage soil saturation.

APPENDIX S3. Experimental flow chart.

APPENDIX S4. Bioclimatic data for each site.

APPENDIX S5. Percentage contribution of each of the 19 bioclimatic variables to PC1.

APPENDIX S6. Clustering of the seven populations according to 19 bioclimatic variables.

APPENDIX S7. Variation among regions and time points in response to treatments.

APPENDIX S8. Heritability and standard errors.

APPENDIX S9. Genetic correlations among traits.

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