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Climate-Related Floral and Vegetative Size Variation in Leptosiphon androsaceus (Polemoniaceae)

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CLIMATE-RELATED FLORAL AND VEGETATIVE SIZE VARIATION IN
LEPTOSIPHON ANDROSACEUS (POLEMONIACEAE)

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Aggie Morrow

August 2012

The Designated Thesis Committee Approves the Thesis Titled

CLIMATE-RELATED FLORAL AND VEGETATIVE SIZE VARIATION IN
LEPTOSIPHON ANDROSACEUS (POLEMONIACEAE)

By

Aggie Morrow

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

SAN JOSÉ STATE UNIVERSITY

August 2012

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ABSTRACT

CLIMATE-RELATED FLORAL AND VEGETATIVE SIZE VARIATION IN
LEPTOSIPHON ANDROSACEUS (POLEMONIACEAE)

by Aggie Morrow

Phenotypic plasticity in plants is a vital adaptive response to changing environmental conditions. Floral and vegetative morphology often varies as biotic and abiotic factors vary. Variation is the basis of evolution, and natural selection, acting on trait variation, can lead to speciation. For this study, floral and vegetative character traits of false babystars (*Leptosiphon androsaceus*, Polemoniaceae) were analyzed within four populations at Henry W. Coe State Park in 2011. Traits were measured to assess changes in size along a moisture availability gradient. Stable carbon isotope ratio ($\delta^{13}C$) samples were collected to measure integrated water-use efficiency (WUE) as it related to precipitation and floral size. Sizes of floral and vegetative characters varied significantly between the drier and wetter regions. Floral and vegetative character sizes for *L. androsaceus* decreased from the wetter to the drier region. The ratio of floral area/leaf area increased as precipitation decreased, suggesting that leaves may have incurred a physiological cost from floral growth within the drier region. A difference in WUE, as it related to trait size and precipitation, was not found for this study year. These results provide evidence that floral and vegetative characters of *L. androsaceus* vary in size in response to water-limiting conditions.

ACKNOWLEDGEMENTS

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INTRODUCTION

Many factors act on plants to affect their growth, morphology, and
physiology. Plants are complex organisms that are constantly being shaped by
environmental forces. These environmental pressures act on phenotype,
directing morphological and physiological variation in plants, establishing the
mechanisms for adaptation and natural selection. Changes in floral and
vegetative characters can be driven by plasticity, pollinators, herbivores,
temperature, and moisture availability (Stebbins, 1970; Schlichting and Pigliucci,
1998; Galen, 1999; Harder and Barrett, 2006). Assessing phenotypic change as
it relates to environmental factors enhances our understanding of variation in
plants.

Plants have enormous capacity for developmental plasticity, and variation
in physiological and morphological traits can result from the interaction between
an organism's genotype and the environment (Schlichting, 1986). Variation in
plants is not always adaptive, and changes in morphology can occur by chance
through changes in the frequencies of alleles in a population due to genetic drift.
Trait variation in plants may also result from plants acclimating to biotic and
abiotic shifts in the environment. Phenotypic plasticity allows an organism to
modify its physiology and morphology in response to ever shifting environmental
conditions. Traditionally, ecologists have focused on pollinators and herbivores
as the primary forces driving variation in floral traits, with these studies
dominating the literature. Studies involving changes in floral and vegetative traits

due to abiotic influences, such as moisture availability, have increased in recent years, yet the forces by which the abiotic environment affects trait variation are not fully understood.

Biotic selection on floral traits

For outcrossing plants that rely on animal pollinators for reproduction, increasing pollinator visitation through floral attractiveness is crucial to the pollination process and reproductive success. Biotically driven variation in floral phenotype is largely mediated by pollinator preference for floral scent, color, and corolla display. The evolution of floral scent has allowed plants to attract animal pollinators that are nocturnally active, such as bat and moth pollinators, to mimic mating pheromones emitted by insects, and target scent preferences of specific pollinators (Galen and Kevan, 1983; Chess et al, 2008; Raguso, 2008). The release of floral scents, which are predominantly a mix of volatile secondary plant metabolites, typically corresponds with a flower's optimal pollination stage (Larcher, 2003).

Plants also use floral color to attract potential pollinators. Floral color increases a plant's attractiveness to pollinators and serves, partially, as a cue for nectar reward (both quantity and quality) (Meléndez-Ackerman et al, 1997). Flower color can be an attractant for a general array of pollinators and plant-specific pollinators. Color can serve as a guide for long distance detection by possible pollinators as well (Sutherland and Vickery, 1993). Floral color has

evolved in many species due to pollinator preference, e.g., snapdragon (*Antirrhinum*, Scrophulariaceae; Jones and Reithel, 2001; Odell et al, 1999), monkeyflower *(Mimulus* spp., Phrymaceae; Schemske and Bradshaw, 1999), and columbine (*Aquilegia* spp., Ranunculaceae; Hodges et al, 2003).

The role that pollinators play in driving floral size variation has been well documented (Galen and Stanton, 1989; Campbell, 1996; Totland, 2001; Elle and Carney, 2003). Studies have shown that selection for flower size occurring within species is partially mediated by pollinator preference for larger flowers. For example, Conner and Rush (1996) tallied pollinator visitation to flowers of wild radish (*Raphanus raphanistrum*, Brassicaceae) based on corolla size in an experimental garden and manipulative treatment. Flower size was found to have a significant impact on flower visitation rates by insects in both the experimental garden and manipulative treatments, with larger flowers attracting more pollinator visits than smaller flowers.

Floral variation can also be driven by conflicting forces due to pollinator and predator preferences. Trade-offs in corolla size can occur when floral morphology directed at pollinator attraction increases reproductive fitness, while larger flowers inadvertently attract floral and seed predators, thereby reducing reproductive fitness. Many experimental studies have documented the impact of pollinators and herbivores on phenotypic variability in flowers (Stebbins, 1970; Cresswell and Galen, 1991; Campbell et al, 2002; Elle and Hare, 2002; Glaettli

and Barrett, 2008; Parachnowitsch and Kessler, 2010). Cariveau et al (2004)

studied the simultaneous impacts of pollinators and predators (plume moth and

Phytomyza species of fly larvae) of flowers and seeds on the morphology of

paintbrush (*Castilleja linariaefolia*, Scrophulariaceae) in the Rocky Mountains.

They found that predation on calyces and seeds affected flower number, calyx

length, seed set, and plant height. Although the flowers of *C. linariaefolia* are

small and inconspicuous, the calyces are large, bright red, and showy, which are

selected for by pollinators. Florivores and seed predators that visited *C.*

linariaefolia reduced the fitness of plants with showy displays, thereby affecting

selection on larger floral organs.

Variation in vegetative traits in relation to the abiotic environment

Plants adapt to drought stress in a number of ways, including limiting

growth, which results in smaller vegetative size. Decreasing the surface area of

stems and leaves reduces the amount of water lost from the plant's surface.

Growth depends mostly on cell expansion, which is controlled by water and

carbon inputs, and acclimation to limited water availability can result in a

reduction in cell expansion and development (Taiz and Zeiger, 2002; Mitchell et

al, 2008; Pantin et al, 2011). Studies of plant responses to changes in the

environment have shown a size reduction in vegetative characters for plants

growing in moisture-limiting conditions (e.g., Clausen et al, 1941; Hiesey et al,

1942; Parkhurst and Loucks, 1972; Dudley, 1996; Chaves et al, 2003; Heschel and Riginos, 2005).

One of the first studies to show plastic variation in vegetative size in response to changes in elevation and climate was conducted by Clausen et al (1941). Genetically identical clones of common cinquefoil (*Potentilla glandulosa*, Rosaceae), yarrow (*Achillea borealis,* Asteraceae), and common yarrow (*Achillea lanulosa*, Asteraceae) were grown in common gardens at three California locations. Experimental plots established at sea level, mid-elevation foothills, and at the crest of the Sierra Nevada displayed drastically different levels of vegetative growth. Plants grown at sea level and mid-elevations grew to more than twice the size of clones grown at the alpine stations. Plants grown at the lowest elevation were typically taller and more robust than clones transplanted to common gardens at mid-elevation and at timberline locations (Clausen et al, 1941; Hiesey et al, 1942). Due to the moderate temperatures and greater moisture availability, plants at sea level grew taller and fuller than plants grown at higher elevations. The use of clones in the Clausen et al (1941) study provided definitive evidence of the plasticity in trait morphology that is possible when plants respond to changing abiotic conditions.

Abiotic environmental factors have also been shown to decrease plant growth in *Metrosideros polymorpha* (Myrtaceae; Cordell et al, 1998) and twisted acacia *(Acacia raddiana*, Mimosaceae; Ward et al, 2012). In Hawaii, *M.*

polymorpha occupies a wide range of habitats and shows high morphological diversity throughout its range. In a study by Cordell et al (1998), character variation in *M. polymorpha* was analyzed at different elevations with varying precipitation. Vegetative measurements from field and greenhouse grown plants showed a reduction in foliar size with increasing elevation and decreasing moisture. Similarly, in a study of *A. raddiana* in the Negev desert of Israel, vegetative traits were measured after being grown under different watering regimes (Ward et al, 2012). Vegetative characters grew smaller in the low water treatment and larger in the high water treatment.

Variation in floral traits in relation to moisture availability

Floral size variation due to abiotic forces, such as moisture limitation, can elicit a change in phenotype that is comparable to pollinator driven variation in flower size. During growth and maintenance flowers use large amounts of water. Water lost through transpiration can be high in flowers because they lack control of their stomata (McDowell and Turner, 2002; Feild et al, 2009). Therefore, smaller flowers, which require less water investment from the plant, will be more beneficial in arid environments. In numerous field and greenhouse studies, floral size has been shown to decrease when water availability was reduced (Galen et al, 1999; Jonas and Geber, 1999; Elle and Hare, 2002; Eckhart et al, 2004; Elle, 2004; Fabbro and Körner, 2004; Hughey and Lambrecht, 2007; Lambrecht and Dawson, 2007). Variation in floral size has been demonstrated along naturally

occurring moisture availability gradients for a number of species. For example

floral traits of true babystars (*Leptosiphon bicolor*, Polemoniaceae; Lambrecht, In

press) and rosemary (*Rosmarinus officinalis*, Lamiaceae; Herrera, 2005) were

shown to vary with different levels of rainfall. Floral traits of *L. bicolor* growing in

separate populations at Henry W. Coe State Park in the coast range of Central

California decreased in size as precipitation declined (Lambrecht, In press).

Herrera (2005) studied floral size in populations of *R. officinalis* along a rainfall

gradient in Spain. Plants within mountain habitats grew the largest flowers,

lowland flowers were intermediate in size, and flowers from plants growing in the

drier coastal habitat grew the smallest.

Plastic responses to limited resources were also displayed in greenhouse

studies where artificially induced drought treatments resulted in decreased flower

size. In a study of fireweed (*Epilobium angustifolium*, Onagraceae; Carroll et al,

2001) plants were grown in a greenhouse under well-watered and drought

treatments. Plants treated with limited water grew 33% smaller flowers. A

greenhouse study by Caruso (2006) analyzed changes in floral character size in

the perennial wildflower great blue lobelia (*Lobelia siphilitica*, Lobeliaceae).

Lobelia plants were also grown under either a well-watered or a drought

treatment, showing smaller floral character growth within the drought treatment.

Brock et al (2009) also found that floral characters of mouse ear cress

(*Arabidopsis thaliana*, Brassicaceae) decreased in size when precipitation

decreased in a common-garden experiment and under drought treatment in a

greenhouse. These studies demonstrated the advantage that reduced flower size imparts to plants growing in drier conditions. Floral growth is water intensive, and a reduction in floral size can be an adaptive strategy utilized by plants growing in xeric habitats to increase survival. The floral size variation demonstrated in these experiments may be due to a plastic response to decreased moisture availability, a strategy for coping with limited resources, or a combination of plasticity and adaptation.

Possible mechanisms for variation in floral traits in relation to moisture availability

The underlying mechanisms by which plants divert resources and maintain floral growth are important in understanding floral size variation as it relates to water supply. Conflicting selection on floral size, mediated by selective forces operating in opposing directions, can result in floral size variation within species. Corolla size differences in alpine skypilot (*Polemonium viscosum*, Polemoniaceae; Galen, 1999, 2000) are driven by opposing environmental forces. Pollinators of *P. viscosum* have been shown to favor larger flowers over smaller flowers. Floral size tends to be directly correlated with nectar volume, which is highly rewarding to pollinators, reinforcing pollinator selection for larger flowers (Cresswell and Galen, 1991). Flowers of *P. viscosum* grew larger when rainfall was abundant, yet in drier environments floral growth decreased even though pollinators preferentially visited flowers with larger corolla displays, thereby increasing floral variation.

Plants often employ overlapping strategies for dealing with environmental conditions where resources are limited. Methods of drought escape and drought avoidance utilized by plants may function in concert to increase survival in dry environments. Plants may escape dehydration by growing small and fast before the driest stage of the growing season (Heschel and Riginos, 2005; Sherrard and Maherali, 2006). This strategy of rapid growth and reproduction, and associated reduction in floral size, may occur before or early in the decline of water availability. Drought avoidance can also be achieved through a physiological response to decreased moisture availability. Plants resist desiccation when water is limited by reducing stomatal conductance, thereby decreasing water loss through transpiration (Kemp and Culvenor, 1994; Mitchell et al, 2008). Plants maintain low photosynthetic activity and growth rate during episodes of drought, decreasing the demand for carbon and water resources that are needed for larger floral and vegetative growth (Thomas, 1997; Galen, 1999; Correia et al, 2001).

Vegetative growth in arid environments is mediated by limited water uptake and mechanisms that prevent water loss. Regulation of CO_2 gas exchange and water lost through transpiration are controlled by foliar stomata responding to internal and external cues. Stomatal closure is triggered by decreased foliar water potential, reduced cell turgor, and low atmospheric humidity (Galen, 1999; Chaves et al, 2002). When the availability of moisture declines, plants close their stomata to limit water loss, but also reduce CO_2

uptake in the process. The ratio of carbon uptake to water lost through transpiration is a measure of a plant's water-use efficiency (WUE). Water-use efficiency in plants may be measured using instantaneous measurements of gas exchange, or inferred from stable carbon isotope ratios, which provide a more integrative measure. There are two stable isotopes of carbon that occur naturally in our atmosphere, with ^{12}C making up 98.9%, and ^{13}C making up 1.1% of atmospheric CO_2 (Farquhar et al, 1989). During the carboxylation stage of the Calvin Cycle of photosynthesis, the enzyme rubisco (ribulose-1,5 -bisphosphate carboxylase oxygenase) discriminates against ^{13}C in favor of the lighter ^{12}C. When intracellular CO_2 concentrations drop due to stomatal closure, the available ^{12}C is assimilated first. When that supply is gone, ^{13}C is assimilated. Therefore, as stomata close, the amount of ^{13}C assimilated into leaf tissue increases. Measuring carbon isotope levels within photosynthetic plant tissue can be used as an indication of long-term patterns of stomatal closure and dehydration avoidance (Geber and Dawson, 1990; Chaves et al, 2003). Water-use efficiency has been studied in relation to moisture availability and vegetative morphology, and increased rates of WUE have been detected in drier environments. Studies have shown that smaller plant size, resulting from low moisture availability, was associated with higher levels of WUE (Dudley, 1996; Martin and Thorstenson, 1988; Caruso et al, 2005).

Floral size reduction in dry environments is due to a whole plant response to the higher levels of water needed for floral growth and maintenance. Water is

essential for floral growth and function, and large amounts of water can be lost from flowers (Galen et al, 1999; McDowell and Turner, 2002; Feild et al, 2009). Water cannot be taken up by flowers directly, and floral organs lack the ability to control water loss through their stomata. The water needed for floral function is supplied directly from vegetative structures (de la Barrera and Nobel, 2004; Galen, 2005; Feild et al, 2009), and the amount of water supplied from vegetative organs affects floral growth, maintenance, and turgidity. The inability to capture water and the lack of control mechanisms to limit water loss by flowers makes floral growth very costly to the whole plant. Growth and maintenance of larger flowers in water-limited environments is physiologically more costly to plants than those in more mesic environments (Galen et al, 1999; Galen, 2005). Therefore, a reduction in floral surface area reduces the loss of water through floral transpiration and increases the conservation of a plant's water supply in dry environments (Galen, 1999; Campbell and Reece, 2002).

During each stage of floral and vegetative growth plants respond to changes in environmental conditions. Variation in floral size displayed in dry environments results from the physiological interaction between flowers and vegetative structures. For example, in *Polemonium viscosum*, Galen et al (1999) found that flowers grew smaller in the drier krummholz environment as opposed to those growing in the wetter tundra habitat. Results of a potometer experiment showed larger flowers required more water during floral growth and maintenance than did smaller flowers. Their findings suggest that floral maintenance reduces

the amount of water available to vegetative structures. Gas exchange measurements displayed a correlation between stomatal closure due to floral water loss, and rates of foliar photosynthesis. Larger flowered plants that grew under drier conditions had lower rates of photosynthesis than did plants with smaller flowers growing under the same conditions. Additionally, smaller flowers showed greater cell densities, which may have resulted in cells using less water to maintain cell turgor. McDowell and Turner (2002) found that reproduction in trailing blackberry (*Rubus ursinus*, Rosaceae) affected photosynthetic capacity. Plants of *R. ursinus* displayed lower leaf water potential and reduced stomatal conductance during reproduction as compared to the closely related, invasive species Himalayan blackberry (*Rubus discolor*, Rosaceae). The reduction in water potential was associated with transpirational water loss from reproductive structures.

The effects of floral maintenance and reproduction on vegetative physiology can result in lowered water potential, reduced photosynthesis, and reduced carbon uptake in foliar structures. Leaves that decrease water loss by closing their stomata might compensate for the transpiration of water from flowers. Assessing the relationship between WUE and floral and vegetative size reveals the floral costs imposed on the whole plant when water availability is low. Lambrecht and Dawson (2007) sampled plants growing in populations at three field sites in Oregon that varied in rainfall and soil moisture content. Floral size measurements were made on a range of native and non-native plant species.

Plants showed an increase in WUE with increasing flower size, suggesting that leaves were closing their stomata to compensate for floral water loss and its effects on overall plant water balance. Water-use efficiency, as it relates to morphological trait size, was also assessed in Lambrecht's (In press) study of *Leptosiphon bicolor*. Water-use efficiency increased as flower size increased. The increase in WUE detected in this study was particularly evident in drier environments during dry years. Within the drier sites, floral area was found to be greater than leaf area, implying a whole plant effort to control transpiration and limit water loss.

The current study is part of a long-term study of false babystars (*Leptosiphon androsaceus*, Polemoniaceae) in Henry W. Coe State Park, Morgan Hill, CA, that was started in 2005 by Dr. Susan Lambrecht (San José State University). Topography varies throughout Henry W. Coe State Park, with high ridges (600 to 900 m in elevation) divided by steep canyons and intermittent valley bottoms. A natural precipitation gradient occurs from the western region of the park to the eastern region. The eastern region is situated in the rain-shadow of the coastal range and receives less precipitation than in the west. The goal of this study, as a part of the multi-year study, was to assess variation in floral and vegetative morphology of the annual wildflower *L. androsaceus* within a single year. This insect-pollinated species is in a genus that has shown high levels of character variation in relation to climate (Hughey and Lambrecht, 2007; Lambrecht, 2010), making it an ideal plant for studying precipitation related trait

variation. In addition, the distribution of *L. androsaceus* along a natural precipitation gradient accommodates the study of climate-dependent trait variation. Within study populations previously established by Dr. Lambrecht along the precipitation gradient, I analyzed floral and vegetative traits to determine whether character size varied in relation to moisture availability. In addition, I evaluated integrated water-use efficiency in relation to floral size, which can be an indicator of floral water costs and leaf-level water control during flowering.

MATERIALS AND METHODS

Study area

Data were collected from four field populations at Henry W. Coe State Park (Coe), from April through June, 2011. Coe is located in the Diablo Range east of Gilroy, California. The park is the largest state park in northern California, consisting of 35,000 ha. Plant communities within the park include grassland, marsh, riparian, chaparral, oak woodland, and mixed coniferous forest.

Two populations of the native plant *Leptosiphon androsaceus* were studied within the drier, eastern region of the park, and two populations were studied within the wetter, western region of the park. The western region of Coe receives an average of 30% more precipitation per year than the eastern region, which is situated in the rain shadow. Western populations are located ~ 0.8 km apart and are named Domino and Bobcat. The Domino population is located in

an oak woodland at 731 m a. s. l. (37°07.260' N, 121°26.884' W). The Bobcat

population lies along a ridge at 740 m a. s. l. (37°07.641' N, 121°27.201' W) in an

oak woodland. Populations within the eastern region are named Mustang and

Woodpecker and are situated ~ 3 km apart. The Mustang population is located at

570 m a. s. l. (37°10.687' N, 121°22.309' W) in a dry, exposed, chaparral

community. The *L. androsaceus* population known as Woodpecker grows in an

oak woodland and is located at 340 m a. s. l. (37°09.717' N, 121°23.701' W).

Abiotic measurements

Soil moisture content and soil texture were measured to determine

whether there were any soil differences among the four sites. Volumetric soil

moisture content was measured from five permanently marked locations within

each site during four data collection visits spanning four weeks. Soil moisture

readings were taken at a depth of 15 cm, which corresponds to maximum rooting

depth of *L. androsaceus*, using a time-domain reflectometry probe (Field Scout

TDR 200, Spectrum Technologies, Plainfield, IL).

Tests were completed at each site to determine soil texture using the

United States Department of Agriculture - Natural Resources Conservation

Service (USDA – NRCS) texture-by-feel method (USDA – NRCS, 2012). Soil

samples from each population were collected and taken to the lab for further

analysis. Samples were mixed with ammonium hydroxide and water, agitated for

twenty minutes, and then left to settle. After three days the percent by weight of

the primary soil separates of sand, silt, and clay were calculated. Percent ratios of soil separates were charted on the USDA - NRCS established Soil Texture Triangle (USDA – NRCS, 2012).

Slope and aspect measurements for the four study locations were completed to assess the micro-environment in which the separate populations of *L. androsaceus* grow. The slope and aspect of all four research sites were recorded using a clinometer, and standard compass.

Average annual rainfall was determined to compare precipitation across the two study regions of Coe. Rainfall averages for the study year (October 2010 through September 2011) were estimated from Santa Clara Valley Water District rainfall gauges stationed throughout Coe (ALERT Precipitation Gauge Information, 2012). Analyses of precipitation from 2005 – 2009 by S. Lambrecht have established that these populations occur along a moisture gradient.

Character measurements

Leptosiphon androsaceus is a native California annual, with palmate leaves having oblanceolate to linear lobes situated in multiple leaf pairs along the main stem**.** The inflorescence is umbel-like with many long-tubed flowers in a head. The salverform corolla is typically white to pink or lavender with lobes measuring 5-10 mm long (Hickman, 1993). The Polemoniaceae family has recently been subjected to reorganization to ensure monophyletic grouping within genera. The genus *Linanthus*, the third largest in the family, has been split into

two major clades: *Linanthus* and *Leptosiphon* (Bell and Patterson, 2000). The

species *androsaceus* was previously classified within the *Linanthus* genus, but

has recently been reclassified as a species of *Leptosiphon*. *Leptosiphon*

androsaceus is dispersed widely throughout California and is commonly found

growing in grassland, oak woodland, coastal scrub, and chaparral communities.

Within each of the four populations, floral and vegetative sizes were

measured on 30 randomly selected plants (*n* = 120 for the four populations

combined). Plants were selected when at least three flowers in the umbel-head

were fully opened. Floral heads with fewer than three opened flowers or more

than two older, withering flowers were avoided, as these were not considered to

be at the peak of reproduction. Floral measurements, which were made with a

caliper to the nearest 0.5 mm, included corolla diameter (measured across the

open corolla face), individual petal lobe length and width (averaged from three

petal lobes on an individual flower in the center of the umbel head), corolla tube

length (measured from the juncture of the involucre and corolla tube to the base

of the corolla face), calyx length (measurement of the longest sepal), and stigma-

to-anther distance measured from the middle of the anther head to the center of

the stigma (Fig. 1). These floral traits included those that are most commonly

used to distinguish between species of *Leptosiphon* and, therefore, should have

some genetic basis.
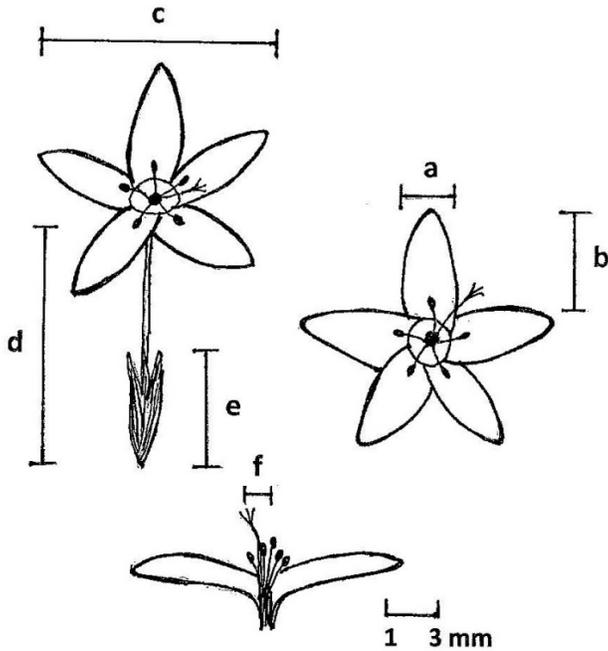
FIG. 1. Diagram of *L. androsaceus* floral characters measured. (a) Petal lobe width, (b) petal lobe length, (c) corolla diameter, (d) corolla tube length, (e) calyx length, and (f) stigma-to-anther distance.

Vegetative measurements included leaf number (the number of leaf whorls along the stem below the umbel, excluding cotyledons), lobe number (the number of lobes in the uppermost cauline whorl), leaf length (length of the longest lobe on the palmate leaf), and vegetative stem height measured from the soil surface to the base of the umbel (Fig. 2).
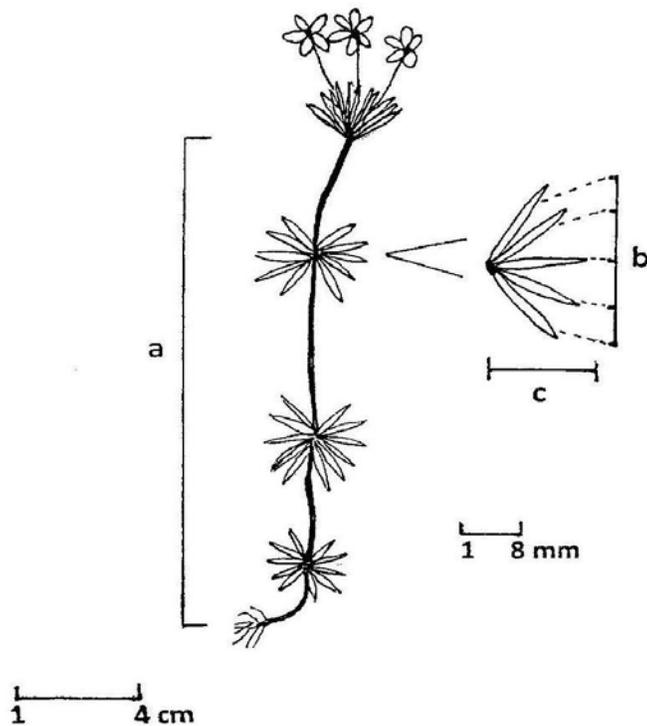
FIG. 2. Diagram of *L. androsaceus* vegetative characters measured. (a) Vegetative stem height (cm), (b) leaf lobe number, and (c) leaf lobe length (mm). The scale on the left applies to vegetative stem height (a), while the scale on the right applies to leaf lobe length (c).

Measurements were made with calipers to the nearest 0.5 mm, except vegetative stem height, which was measured with a standard ruler to the nearest 0.5 cm. Floral area and leaf area were calculated using regression equations developed between area and size measurements by Dr. S. Lambrecht (Lambrecht et al, In prep) during her previous years of work on *L. androsaceus* within these populations at Coe. Leaf area (from the uppermost pair of leaves) and corolla area were previously measured in the field using a portable leaf area analyzer (CID 202: CID Analytical, Camas, WA) in conjunction with the same

caliper size measurements made in this study. In the current study, the caliper-measured traits were used in the regressions to estimate floral and leaf area of the study plants. Using the regression equations to estimate floral and leaf area from measured traits eliminates the need to destroy plants in the field populations. Floral area was calculated using the measured corolla diameter and petal lobe width (floral area = 1.099 – (2.737 x (1/corolla diameter x petal width))) for each plant. Leaf area was calculated using leaf lobe length, lobe number, and leaf number for the western region (leaf area = (-.2382 + (.011 x leaf length x leaf lobe number)) x leaf number) and the eastern region (leaf area = (-.0847 + (.0056 x leaf length x leaf lobe number)) x leaf number). The calculated floral area value was divided by the calculated leaf area value to establish the floral area/ leaf area ratio (FA/LA).

Stable carbon isotope measurements

Foliar stable carbon isotope ($\delta^{13}C$) ratio, or the ratio of $^{13}C/^{12}C$ in photosynthetic tissue, was measured from randomly selected plants to infer integrated water-use efficiency (WUE). Stable carbon isotope levels are calculated as the ratio of $^{13}C/^{12}C$ in photosynthetic tissues relative to that of a standard, which is fossil belemnite from the Pee Dee limestone formation in South Carolina. Isotope ratios are calculated on a per mil (‰) scale:

$\delta^{13}C$ ‰ = ($R_{sample}/R_{standard}$ -1) x 1000

(Farquhar et al, 1989).

To obtain $\delta^{13}C$ samples, five plants for which size measurements were

made were collected from each of the four populations (n = 20). Collected plants

were packed in dry ice in the field to prevent desiccation and to slow any

physiological processes, and then taken to the lab at SJSU for preparation.

Samples were dried for 24 h in a drying oven at 70°C. After drying, stem and leaf

tissue were separated from floral and root tissues and ground into a fine powder

using an 8000 M (mix/mill) mechanical ball grinder (Spex CertiPrep, Metuchen,

NJ). Analyses of $\delta^{13}C$ ratios were done on 1- 2 mg subsamples using a Delta-V

Advantage Isotope Ratio Mass Spectrometer operating in continuous-flow mode

with a Costech elemental analyzer inlet system at the Facility for Isotope Ratio

Mass Spectrometer (FIRMS, University of California, Riverside).

Data analyses

In order to test for floral, leaf, and plant size differences between the

wetter and drier regions, character means were analyzed using an analysis of

variance (ANOVA). Populations (Domino, Bobcat, Mustang, and Woodpecker)

were nested within their respective regions to test for differences in average

character sizes between populations. Due to simultaneous testing of multiple

variables, probability values were adjusted using the Bonferonni adjustment to

reduce the likelihood of erroneously rejecting a true null hypothesis (Rice, 1989).

Tukey-Kramer post hoc tests for pairwise comparisons of populations were

completed to identify which populations were significantly different from one

another. An ANOVA test was used to describe differences in mean $\delta^{13}C$ ratios between the wetter and drier regions. Variances in soil moisture content means between populations were also analyzed for significance. Pairwise comparisons of population differences in soil moisture content were tested by the Tukey-Kramer method.

A principle component analysis (PCA) was used to reduce character variables and describe the differences in floral and vegetative character size between the two regions. Separate analyses were done for each of the regions to establish which variables described the greatest amount of variation within the wet and dry regions. Components describing the greatest percentage of variance among character traits, within the wetter and drier regions, were calculated with trait measurements and plotted for visual analysis.

The relationships between floral and vegetative characters were described using a simple correlation analysis. Pairwise correlation coefficient values were calculated to evaluate the correlations between measured characters within the separate eco-regions. Character correlations were tested for significance using the Bonferonni method.

Other data analyses were conducted to examine the continuous variation in characters along the moisture gradient. A regression analysis was used to test whether there was a relationship between site moisture and each of the traits of floral area, corolla diameter, leaf area, and FA/LA. Site moisture was a ranking

based on values of precipitation and soil moisture measured in each of the populations since 2005. The populations were ranked from wettest to driest: Domino, Bobcat, Woodpecker, and Mustang.

Least squares linear regression analyses were performed to examine the relationships between $\delta^{13}C$ levels and floral area, corolla diameter, and the FA/LA ratio. Post hoc power analyses of $\delta^{13}C$ samples were conducted to evaluate whether the sample size was large enough to avoid making a Type II error. The post hoc power analysis applied to these data indicated that the sample size was not large enough to insure statistical power. Using a power threshold of 0.80 showed the sample size was too small to find a statistically significant relationship between $\delta^{13}C$ levels and character size. To be sufficiently powerful, a sample size > 1,000 needed to be used to detect effect.

Tests of normality

Statistical test assumptions of normality were assessed by testing skewness and kurtosis, and from the Shapiro-Wilks normality test. Normality was also evaluated visually using histogram and probability plots of variables. Residuals from statistical tests were also subjected to visual examinations for normality. Shapiro-Wilks normality tests revealed that all characters, except leaf number, leaf lobe number, stigma-to-anther distance, leaf area, and FA/LA were normally distributed for the western and eastern regions, and leaf length for the eastern region alone. Non-normal characters had significant W-values of $P <$

0.001. Assessment of normality tests, including visual evaluations, found leaf length, stigma-to-anther distance, leaf area, and FA/LA not sufficiently fitting the normal distribution, therefore these variables were transformed (Fernandez, 1992). Leaf length and FA/LA were log transformed, and stigma-to-anther distance and leaf area were square transformed.

RESULTS

Abiotic measurements

There was a significant difference in average soil moisture content among the four populations ($F_{3,75}$ = 7.86, P < 0.001). Mean soil moisture content readings for the Domino and Bobcat populations within the wetter region were 8.5% and 9.0%, respectively. Within the drier region, soil moisture content for the Mustang population was 8.5%, and was 12.6% for the Woodpecker population. Soil moisture content for the Woodpecker site was significantly higher than that of the other sites (Tukey P = 0.001 as compared to Domino; Tukey P = 0.003 as compared to Bobcat; Tukey P = 0.001 as compared to Mustang; Fig. 3). Rainfall for the study year was 79.88 cm for the wetter region and 57.15 cm for the drier region (ALERT Precipitation Gauge Information, 2012; Table 1).
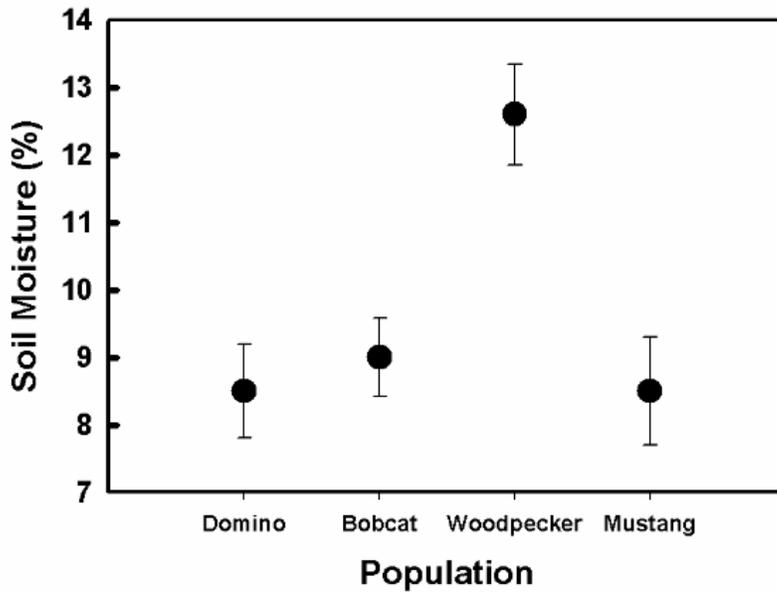
FIG. 3. Volumetric soil moisture content for the four populations at Coe. The Woodpecker population had significantly higher moisture content than the other three populations ($F_{3,75}$ = 7.86, *P* = 0.001 for Domino; *P* = 0.003 for Bobcat; *P* = 0.001 for Mustang).

TABLE 1*. Abiotic conditions for the four populations at Coe. The wetter region includes Domino and Bobcat, and the drier region includes Mustang and Woodpecker.*

Population	Region	Annual precipitation (cm)	Soil Moisture (%; s.e.)	Soil Texture	Slope (%)	Aspect
Domino			8.5 (0.698)	sandy loam	85	40˚
Bobcat	Wetter	79.88	9.0 (0.578)	sandy loam	55	10˚
Mustang			8.5 (0.807)	sandy loam	95	270˚
Woodpecker	Drier	57.15	12.6 (0.745)	sandy loam/ sandy clay loam	60	22˚

Soil texture analyses showed that all four populations have sandy loam soil, as based on the percent by weight of the primary soil separates (Table 2). Sampling soil with the texture-by-feel-method (USDA – NRCS, 2012), revealed sandy loam soil for the Domino, Bobcat, and Mustang populations, and sandy clay loam soil for the Woodpecker population.

TABLE 2. *Percent by weight of primary soil separates for each population.*

Population	% Sand	% Silt	% Clay
Domino	67	27	6
Bobcat	65	30	5
Mustang	64	26	6
Woodpecker	60	33	7

The four population sites varied in relation to slope and aspect. Within the wetter region, the Domino population inhabits a precipitous 80- 90% slope facing east to north-east, and the Bobcat population has a direct north- facing slope that varies between 45% and 65%. Within the drier region, the Mustang population has a very steep slope at approximately 95% that faces due west. The Woodpecker population faces north with a slope varying between 48% in some areas, 50% to 80% in other areas and approximately 95% at a steep road cut face (Table 1).

Comparison of characters between regions in Coe

Statistical analyses revealed significant floral and vegetative character differences between the different regions of Coe (Tables 3, 4 and Fig. 4, 5, 6). From the ANOVA analysis, petal lobe length, petal lobe width (Fig. 4), calyx length, and leaf lobe number (Fig. 5) grew larger on the wetter side than on the drier side of Coe. Leaf area was greater for plants growing within the wetter, western region than for those within the eastern region (Fig. 6 and Table 4). The FA/LA ratio values were greater for plants growing in the drier region than for those in the wetter region of Coe (Fig. 6 and Table 4).

TABLE 3. *Basic statistics for floral and vegetative traits within the wetter and drier regions. Means and standard errors (s.e.)* (n = 60).

Characters	Mean (s.e.) Wetter	Mean (s.e.) Drier
Petal lobe width (mm)	5.5 (0.1)	5.0 (0.1)
Petal lobe length (mm)	8.5 (0.1)	7.9 (0.1)
Corolla diameter (mm)	18.1 (0.3)	17.1 (0.3)
Corolla tube length (mm)	19.4 (0.3)	20.6 (0.5)
Stigma-to-anther distance (mm)	2.0 (0.2)	1.5 (0.1)
Floral area (cm^2)	0.3 (0.01)	0.3 (0.01)
Calyx length (mm)	8.7 (0.2)	7.4 (0.1)
Leaf length (mm)	10.2 (0.3)	10.9 (0.4)
Leaf number	4.0 (0.1)	3.6 (0.1)
Leaf lobe number	6.1 (0.3)	5.0 (0.2)
Leaf area (cm^2)	2.2 (0.3)	0.9 (0.1)
Vegetative stem height (cm)	21.7 (0.6)	21.1 (0.8)
Floral area/leaf area (cm^2/cm^2)	0.2 (0.1)	0.7 (0.1)

TABLE 4. ANOVA tests of morphological characters based on general linear model. Character means were compared between regions (wetter and drier) and between populations (nested within region). n *= 120.* P *–values were Bonferonni corrected. Significant* P *–values are shown in bold. Abbreviations: sq = square root transformed, log = log transformed.*

Characters	Effect	df	MS	*F*	*P*
Petal lobe width	Region	1	6.73	10.45	**0.002**
	Population (region)	2	1.71	2.66	0.005
	Error	116	0.64		
Petal lobe length	Region	1	10.17	9.33	**0.003**
	Population (region)	2	9.25	8.49	**<0.001**
	Error	116	1.09		
Corolla diameter	Region	1	29.30	6.22	0.003
	Population (region)	2	30.79	6.54	**0.002**
	Error	116	4.70		
Corolla tube length	Region	1	37.18	4.51	0.004
	Population (region)	2	107.16	13.00	**<0.001**
	Error	116	8.24		
Calyx length	Region	1	45.16	33.23	**<0.001**
	Population (region)	2	0.84	0.62	0.017
	Error	115	1.36		
Stigma-to-anther distance (sq)	Region	1	0.82	4.50	0.004
	Population (region)	2	0.28	1.55	0.013
	Error	116	0.18		
Floral area	Region	1	0.02	2.50	0.006
	Population (region)	2	0.03	3.33	0.004
	Error	116	0.01		
Floral area/ leaf area (log)	Region	1	20.75	15.42	**<0.001**
	Population (region)	2	2.49	1.85	0.008
	Error	112	1.34		
Leaf number	Region	1	3.67	4.54	0.003
	Population (region)	2	7.70	9.52	**<0.001**
	Error	116	0.81		
Leaf length (log)	Region	1	0.13	2.18	0.007
	Population (region)	2	0.02	0.37	0.050
	Error	116	0.06		

TABLE 4. *Continued*

Characters	Effect	df	MS	F	P
Leaf lobe number	Region	1	38.53	10.94	**0.001**
	Population (region)	2	5.97	1.69	0.010
	Error	116	3.52		
Leaf area (sq)	Region	1	6.83	23.61	**<0.001**
	Population (region)	2	0.65	2.27	0.006
	Error	113	0.29		
Vegetative stem height	Region	1	10.32	0.36	0.025
	Population (region)	2	69.7	2.44	0.005
	Error	116	28.57		
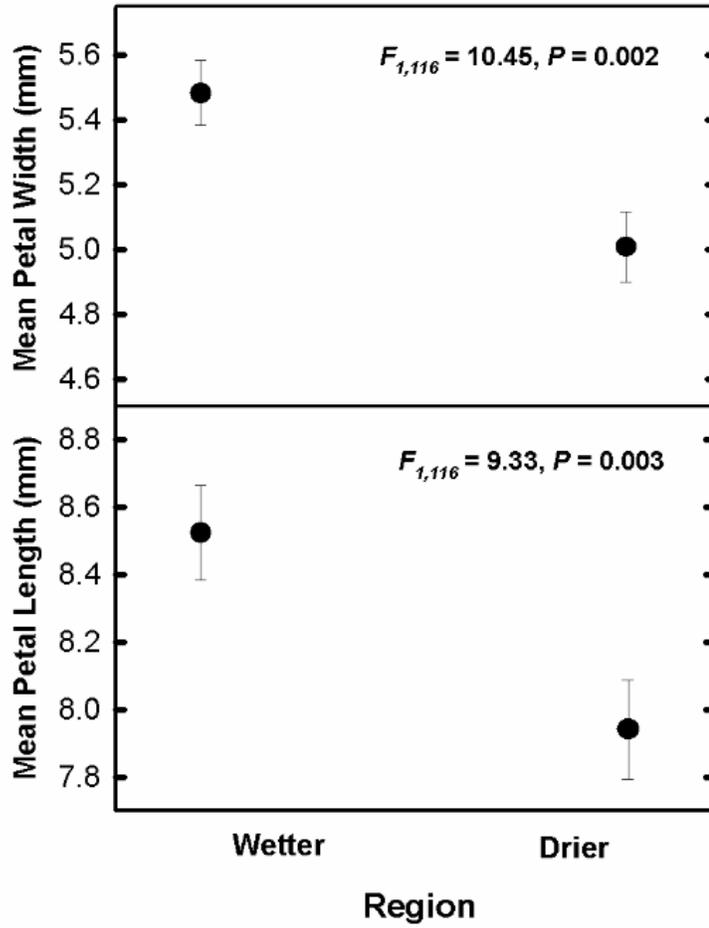
FIG. 4. Floral character means (± s.e.) differing between the wetter and drier regions. The *F* and *P*- values are based on ANOVA (see Table 4).
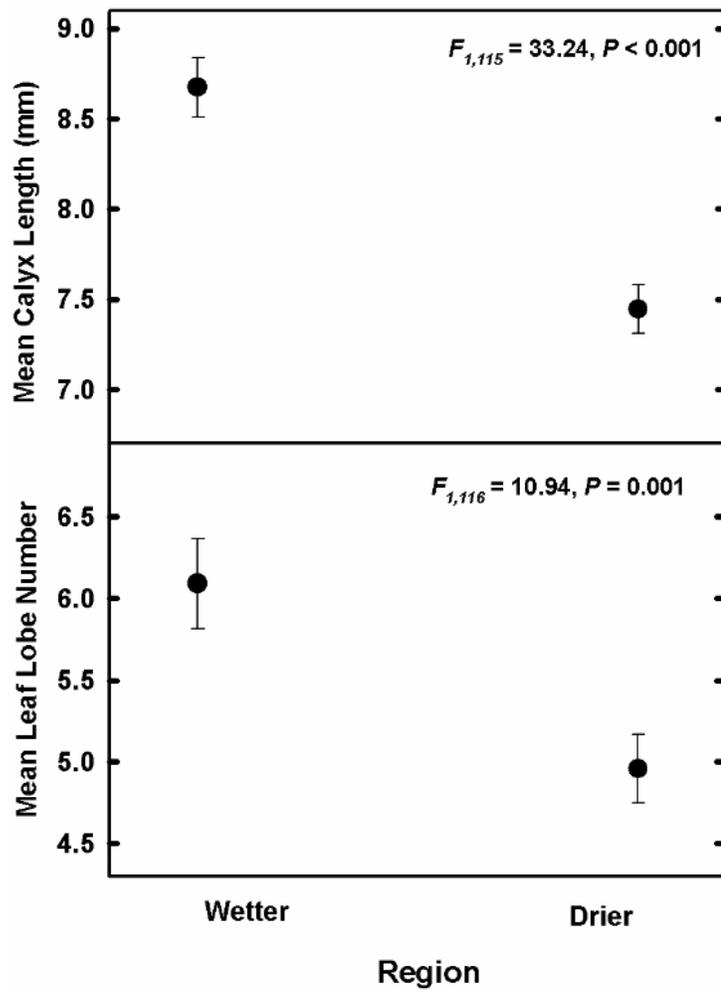
FIG. 5. Leaf and floral character means (± s.e.) differing between the wetter and drier regions. The *F* and *P*- values are based on ANOVA (see Table 4).
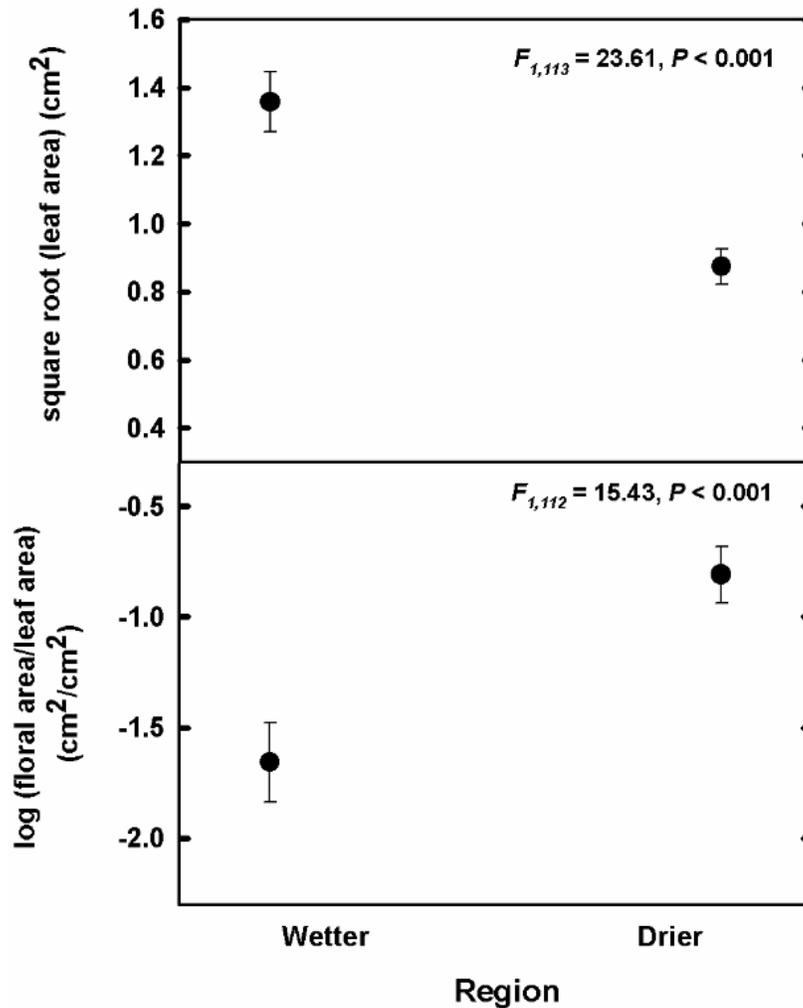
FIG. 6. Floral and leaf area character mean (± s.e.) differences between the wetter
and drier regions. The *F* and *P*- values are based on ANOVA (see Table 4).

Differences in character means between populations were found for plants
at Mustang (the driest, eastern population), which produced shorter petal lobes
($F_{2,116}$ = 8.49, Tukey P < 0.001 as compared to Domino; Tukey P = 0.006 as
compared to Woodpecker) and smaller corolla diameters ($F_{2,116}$ = 6.54, Tukey P
< 0.001 as compared to Domino; Tukey P = 0.02 as compared to Woodpecker)

than plants at either the Domino or Woodpecker populations (Fig. 7 and Table 4).

Corolla tube length was greater for plants at Woodpecker as compared with the

other three populations ($F_{2,116}$ = 13.00, Tukey P = 0.001 as compared to Domino;

Tukey P = 0.001 as compared to Bobcat; Tukey P < 0.001 as compared to

Mustang; Fig. 7 and Table 4). Leaf number was the only vegetative character

that displayed a difference in mean size between populations ($F_{2,116}$ = 9.52, P <

0.001). The average number of leaves per plant within the Mustang population

was lower than the average number of leaves within the other three populations

(Tukey P = 0.007 as compared to Domino; Tukey P = 0.001 as compared to

Bobcat; Tukey P < 0.001 as compared to Woodpecker; Fig. 8 and Table 4).
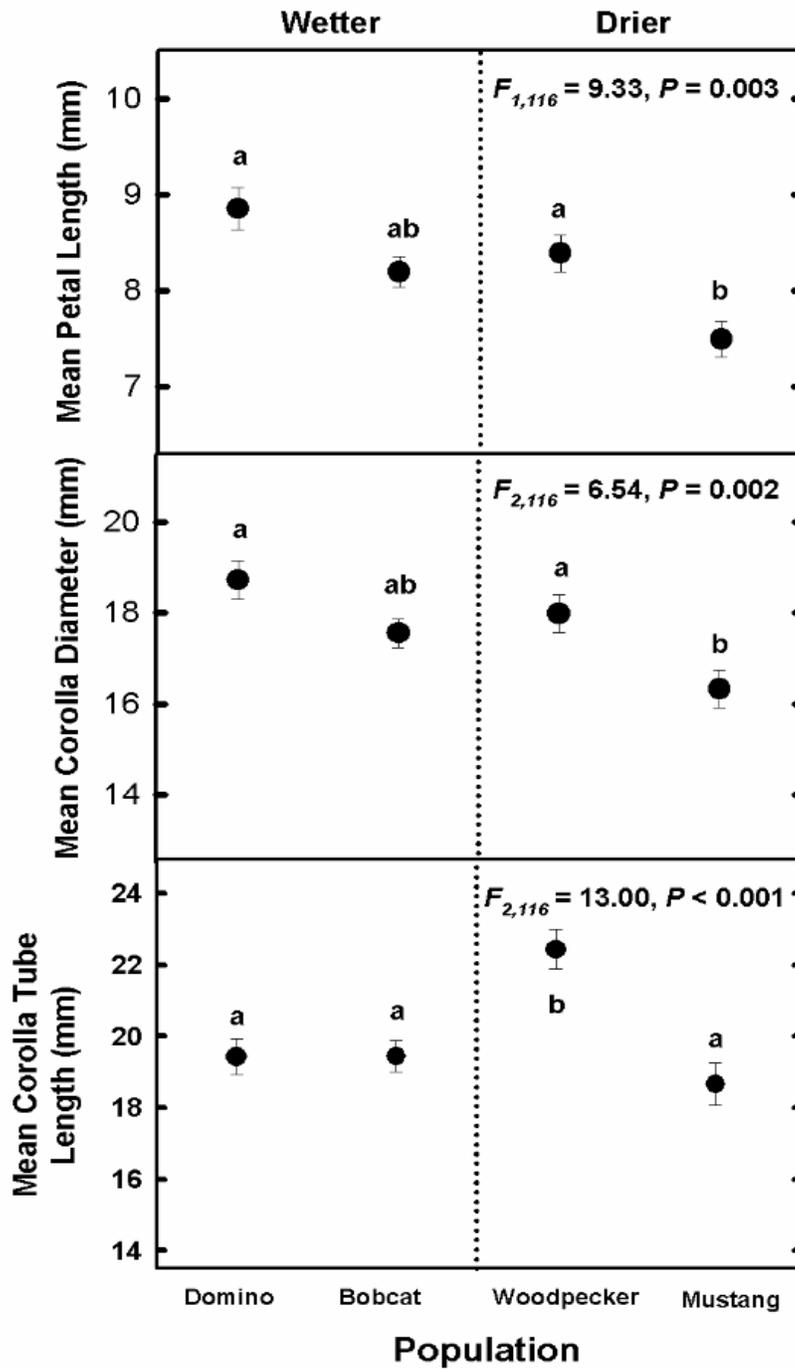
FIG. 7. Floral size (± s.e.) differences among the four populations. The *F* and *P-* values are based on ANOVA (see Table 4). Symbols labeled with different letters are significantly different at *P* ≤ 0.05 (Tukey-Kramer pairwise comparison test).
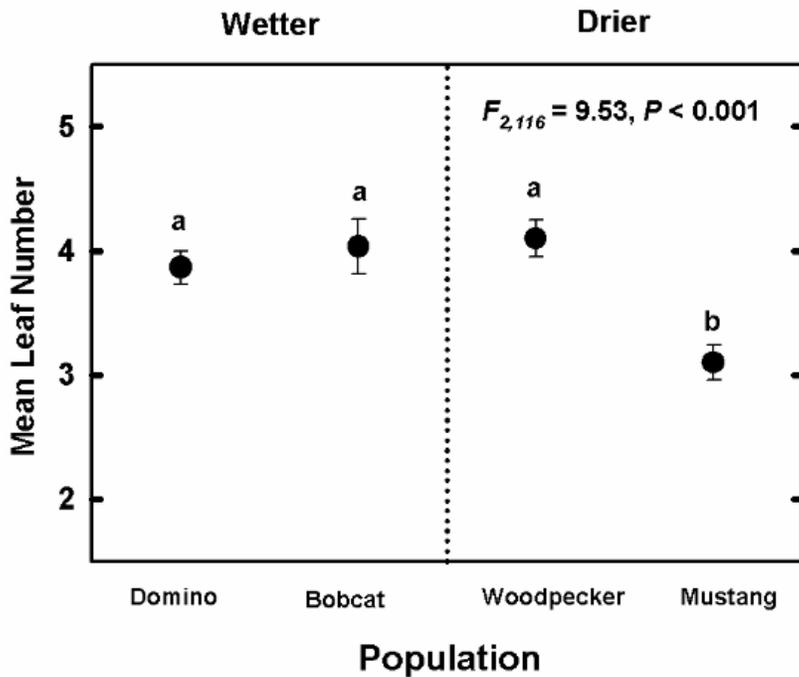
FIG. 8. Leaf number (± s.e.) differences among the four populations. The *F* and *P*- values are based on ANOVA (see Table 4). Symbols labeled with different letters are significantly different at *P* ≤ 0.05 (Tukey-Kramer pairwise comparison test).

The principal components analysis described variation in the variables and helped summarize character differences between the wetter and drier regions. The first component for the wetter region accounted for 35% of the total variance and was highly correlated with leaf lobe length, leaf lobe number, leaf area, and vegetative stem height. Component 2 accounted for 22% of the total variance and was highly correlated with petal lobe length and corolla diameter, while the remaining components accounted for ≤ 13% of the variance each (Table 5; Fig. 9). For the drier region, the first component explained 42% of the

total variance and was highly correlated with petal lobe length, corolla diameter, leaf number, leaf lobe length, leaf area, and vegetative stem height. Component 2 explained 15% of the variance, and the remaining components accounted for ≤ 12% of the variance each (Table 5; Fig. 10).

TABLE 5. *Character loadings on principal components analysis for floral and vegetative characters for the wetter, western and drier, eastern regions. Western region loadings on* components 1 and 2 explain 35% *and* 22% *of variance, respectively. Eastern region loadings on* components 1 and 2 explain 42% and 15% *of variance, respectively.*

Character	Loadings on Component 1 (Wetter)	Loadings on Component 2 (Wetter)	Loadings on Component 1 (Drier)	Loadings on Component 2 (Drier)
Petal lobe width	0.51	0.37	0.68	-0.47
Petal lobe length	0.44	0.83	0.81	-0.42
Corolla diameter	0.46	0.80	0.79	-0.46
Corolla tube length	0.01	0.56	0.56	-0.56
Calyx length	0.45	0.47	0.56	0.01
Leaf number	0.62	-0.45	0.72	0.16
Leaf length	0.86	-0.06	0.71	0.45
Leaf lobe number	0.86	-0.33	0.69	0.44
Leaf area	0.88	-0.39	0.84	0.48
Vegetative stem height	0.79	-0.19	0.71	0.28
Stigma-to-anther distance	0.31	0.04	-0.05	0.34
Floral area	0.15	0.31	-0.03	0.12
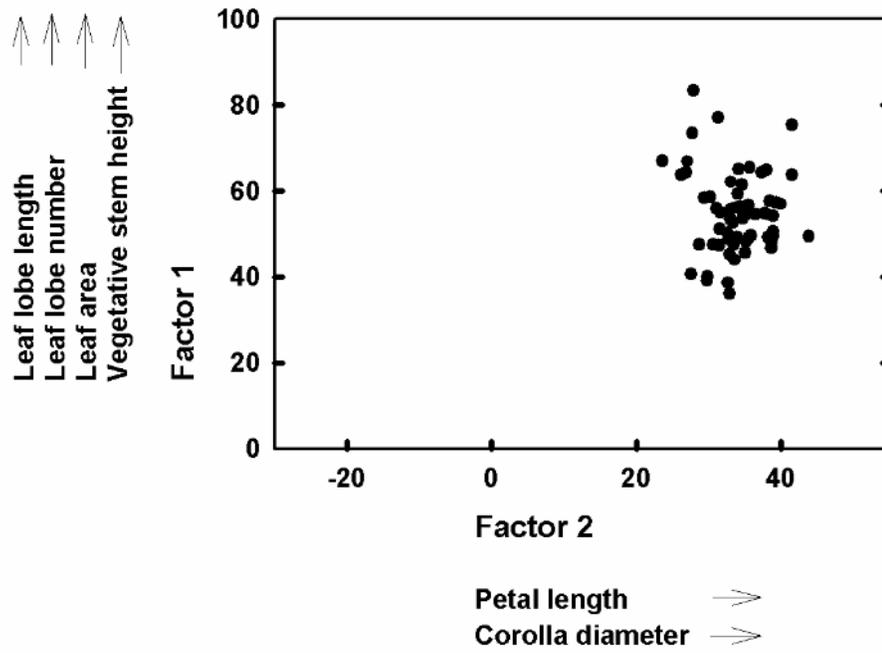
FIG. 9. Scatterplot of principle components for the wetter region of Coe. Factor 1 and factor 2 are labeled with characters having loading scores ≥ 0.70.
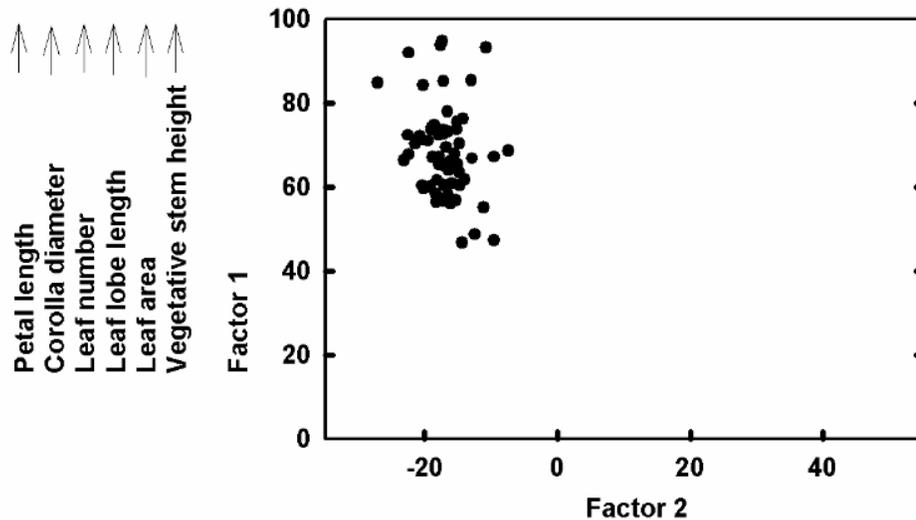
FIG. 10. Scatterplot of principle components for the drier region of Coe. Factor 1 labeled with characters having loading scores ≥ 0.70.

Character correlations

Correlation analyses revealed several positive correlations between measured variables. Correlation results measuring the relationship between variables for the wetter region showed corolla diameter and petal lobe length to be highly correlated ($r = 0.92$, $P < 0.001$; Table 6). There were also significant positive correlations between petal lobe width and petal length ($r = 0.57$, $P < 0.001$; Table 6), and between petal lobe width and corolla diameter ($r = 0.54$, $P < 0.001$; Table 6). Vegetative stem height and leaf characters displayed positive correlations as well. Vegetative stem height was positively correlated with leaf number ($r = 0.51$, $P = 0.002$), leaf length ($r = 0.68$, $P < 0.001$), and leaf lobe number ($r = 0.63$, $P < 0.001$; Table 6). Leaf lobe number was also positively

correlated with leaf number (*r* = 0.61, *P* < 0.001) and leaf length (*r* = 0.72, *P* < 0.001; Table 6).

TABLE 6. *Wetter region character correlations. Correlations among measured floral and vegetative characters. n = 59. Abbreviations: PL=petal length; PW= petal lobe width; CD= corolla diameter; CT= corolla tube length; CL=calyx length; LN=leaf number; LL= leaf length; LO= leaf lobe number; VSH= vegetative stem height, SA= stigma-to-anther distance. P-values have been Bonferroni corrected for multiple comparisons.*

	PW	PL	CD	CT	CL	LN	LL	LO	VSH
PL	0.571*								
CD	0.542*	0.923*							
CT	0.206	0.331	0.272						
CL	0.213	0.445	0.415	0.354					
LN	0.078	-0.090	-0.024	-0.140	-0.029				
LL	0.246	0.264	0.272	-0.043	0.380	0.438			
LO	0.276	0.105	0.158	-0.267	0.124	0.613*	0.720*		
VSH	0.179	0.172	0.166	-0.033	0.263	0.511**	0.685*	0.633*	
SA	0.080	0.092	0.108	0.018	0.180	0.329	0.123	0.181	0.241

*P < 0.001, **P = 0.002

Plants growing in the drier environment displayed more correlations between floral traits than did plants growing in the wetter environment. Similar to plants in the wetter environment, plants in the drier environment displayed a positive relationship between petal lobe length and corolla diameter (*r* = 0.93, *P* < 0.001; Table 7). Other positive correlations observed in the dry environment include petal lobe width with petal lobe length (*r* = 0.65, *P* < 0.001), petal lobe width with corolla diameter (*r* = 0.70, *P* < 0.001), petal lobe width with corolla tube length (*r* = 0.51, *P* = 0.002), petal lobe length with corolla tube length (*r* = 0.62, *P* < 0.001), and corolla diameter with corolla tube length (*r* = 0.60, *P* < 0.001; Table 7). Vegetative stem height and leaf character correlations were

similar for both regions. Vegetative stem height was positively correlated with leaf number ($r = 0.61$, $P < 0.001$), leaf length ($r = 0.53$, $P < 0.001$), and leaf lobe number ($r = 0.52$, $P < 0.001$; Table 7). Leaf lobe number was positively correlated with leaf number ($r = 0.52$, $P < 0.001$) and leaf length ($r = 0.52$, $P < 0.001$; Table 7).

TABLE 7. *Drier region character correlations. Correlations among measured floral and vegetative characters. n = 59. Abbreviations: PL=petal length; PW= petal lobe width; CD= corolla diameter; CT= corolla tube length; CL=calyx length; LN=leaf number; LL= leaf length; LO= leaf lobe number; VSH= vegetative stem height, SA= stigma-to-anther distance. P-values have been Bonferroni corrected for multiple comparisons.*

	PW	PL	CD	CT	CL	LN	LL	LO	VSH
PL	0.645*								
CD	0.695*	0.926*							
CT	0.507**	0.615*	0.597*						
CL	0.332	0.435	0.403	0.192					
LN	0.343	0.471	0.440	0.313	0.301				
LL	0.364	0.383	0.384	0.113	0.413	0.373			
LO	0.213	0.394	0.285	0.225	0.301	0.522*	0.545*		
VSH	0.35	0.376	0.364	0.292	0.308	0.616*	0.531*	0.528*	
SA	-0.052	-0.109	-0.066	-0.152	0.009	-0.14	0.188	-0.049	-0.011

*$P < 0.001$, **$P = 0.002$

Character size and relationship across the precipitation gradient

Regressions between measured characters and sites along the precipitation gradient were used to determine whether character size changed with moisture. The ratio of FA/LA decreased significantly as site moisture increased ($R^2 = 0.07$, $P < 0.004$; Table 8 and Fig. 11). The relationship between leaf area and site moisture was significant, with leaf area decreasing in size from

the wetter to the drier region (R^2 = 0.11, P < 0.001; Table 8 and Fig. 12). There

was no significant relationship between site moisture and floral area, or corolla

diameter (Table 8).

TABLE 8. *Regression analyses of floral and leaf characters to site moisture. Abbreviations: FA = floral area, LA = leaf area, CD = corolla diameter, FA/LA= floral area/leaf area, sq = square root transformed, log= log transformed. Significant* **P***-values shown in bold.*

Relationship	Slope	R^2	P
FA across sites	0.005	0.003	0.548
CD across sites	-0.346	0.028	0.066
LA (sq) across sites	-0.179	0.113	**< 0.001**
FA/LA (log) across sites	0.292	0.070	**0.004**
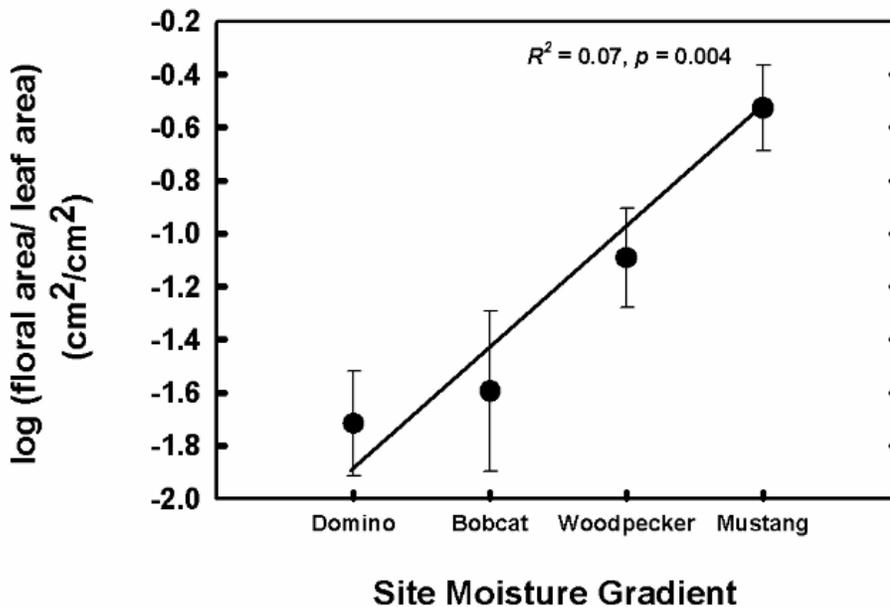
FIG. 11. The relationship between average FA/LA ratios and site moisture. Floral area/leaf area increases from the wetter region to the drier region. Each data point shows the mean (± s.e.) for all values at that population. The regression was fit to a scatter of all data points relative to site moisture rank (from wet to dry).
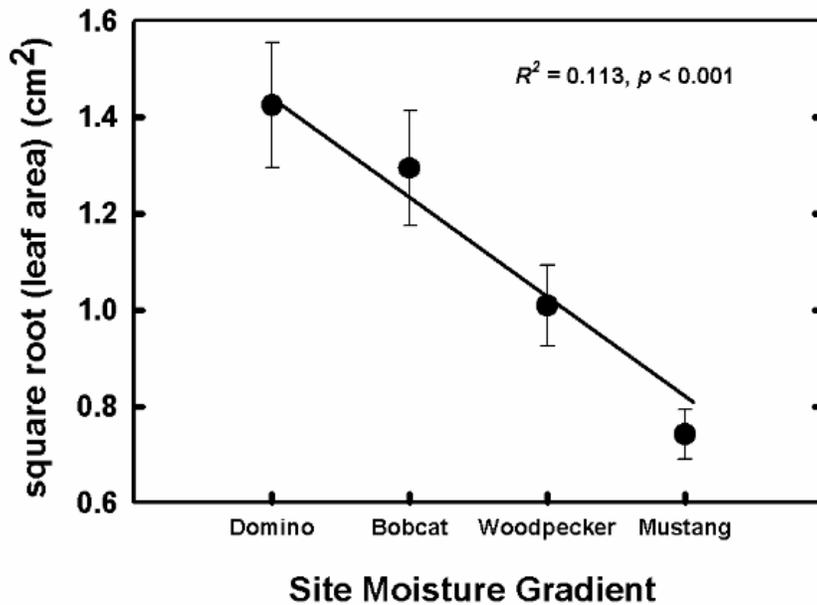
FIG. 12. The relationship between average leaf area and site moisture. Leaf area decreases from the wetter region to the drier region. Each data point shows the mean (± s.e.) for all values at each population. The regression was fit to a scatter of all data points relative to site moisture rank (from wet to dry).

Relationship between $\delta^{13}C$ and floral characters

There was no relationship between $\delta^{13}C$ and floral size traits. There was no evidence of a relationship between $\delta^{13}C$ and FA/LA for the wetter or the drier environment (Table 9). Additionally, there was no relationship between $\delta^{13}C$ and either floral area or corolla diameter for the wetter or drier regions (Table 9).

TABLE 9. *Regression analyses of $\delta^{13}C$ to floral traits. Relationship where the $\delta^{13}C$ ratio is dependent on floral area, corolla diameter, and the floral area/leaf area ratio. Abbreviations: FA = floral area, LA = leaf area, FA/LA = floral area/ leaf area, $\delta^{13}C$ = stable carbon ratio, log = log transformed.*

Relationship	Slope	R^2	P
Wetter region			
$\delta^{13}C$ to corolla diameter	0.017	0.001	0.933
$\delta^{13}C$ to FA	4.111	0.090	0.401
$\delta^{13}C$ to FA/LA (log)	-0.114	0.014	0.765
Drier region			
$\delta13C$ to corolla diameter	-0.081	0.028	0.692
$\delta^{13}C$ to FA	-1.771	0.013	0.789
$\delta^{13}C$ FA/LA (log)	0.093	0.002	0.910

From these results the relationship between the $\delta^{13}C$ ratio and corolla diameter, floral area, and the FA/LA ratio were not significant for plants within the wetter region, or plants within the drier region of Coe. Additionally, an ANOVA analysis showed that there was no significant difference in mean values for the $\delta^{13}C$ ratios between plants from the wetter and drier environments of Coe ($F_{1,16}$ = 2.46, P = 0.14).

DISCUSSION

Floral and vegetative character variation related to moisture availability

Size differences were observed in floral and leaf morphology of *Leptosiphon androsaceus* between the wetter and drier environments of Coe.

Floral size was significantly lower within the drier region compared to the wetter region. The average size of petal lobe width, petal lobe length, and calyx length decreased within the drier region. Size reductions in floral organs displayed within the drier region may be the result of acclimation to water-limited conditions where larger organ size can be too costly to maintain. Flowers use water for organ growth, corolla opening, floral transpiration, and nectar production. The water cost for floral growth and maintenance can be high, and a reduction in floral size in dry environments may reduce floral demand for water uptake. In smaller flowers, tightly packed cells allow turgor pressure to remain high with less water, and this reduced water requirement allows plants with decreased floral area to grow and reproduce under drought conditions (Galen et al, 1999). Similar to these results, many researchers have documented a reduction in floral size under water-limiting conditions (Robertson et al, 1994; Galen, 2000; Carroll et al, 2001; Elle and Hare, 2002; Elle, 2004; Herrera, 2005; Caruso, 2006; Hughey and Lambrecht, 2007; Lambrecht and Dawson, 2007; Halpern et al, 2010; Lambrecht, 2010; Wu et al, 2010).

Differences in mean character size were also found for floral characters among the four populations in Coe. Smaller petal lobe length and corolla diameter were displayed within the Mustang population compared to the other three populations. Plants within the Woodpecker, Domino, and Bobcat populations predominantly grow in oak woodlands, as opposed to the Mustang population, where plants grow on a steep, exposed, west facing slope, in dry

chaparral habitat. The differences in habitat, aspect, and sun exposure between Mustang and the other three populations may have affected the floral size variation detected between populations.

Within the Woodpecker population, corolla tubes grew longer than in the other three populations. Pollinator foraging, proboscis length, and predation can affect floral tube length (Inouye, 1980; Nilsson, 1988; Galen and Cuba, 2001). *Leptosiphon androsaceus* is an insect-pollinated annual (Battaglia and Patterson, 2001), but the abiotic environment may influence corolla tube growth as well. During their previous years studying these populations of *L. androsaceus,* Hughey and Lambrecht (2007) found that pollinators preferentially visited larger flowers within all four populations, and pollinators tended to be of the same type. In addition, corolla tube length did not vary with moisture availability across the moisture gradient (Lambrecht, 2010); therefore, it is unlikely that pollinators affected corolla tube length within the Woodpecker population. The Woodpecker site is located within the drier region of Coe where plants grow in a partially shaded oak understory. Although it is located in the drier region, this population had higher soil moisture content than the other three populations. Higher levels of soil moisture had not been detected in the Woodpecker site, as compared to the other three sites, in our previous years of study. Within the Woodpecker population, grass and herb cover was particularly dense compared to the other three sites for the year of this study. Plants growing below the canopy or within dense neighboring vegetation perceive a reduction in the red to far-red ratio of

incoming light, prompting a shade-avoidance response in the plant. Dense

neighboring vegetation allows more far-red light to pass through than red light,

triggering a plant response resulting in stem elongation, accelerated flowering,

and increased floral growth (Smith and Whitelam, 1997; Devlin et al, 1999). The

increased corolla tube growth displayed within the Woodpecker population may

have been a shade-avoidance response initiated by the abundance of tall

grasses that grew in proximity to *L. androsaceus* plants.

Leaf traits also displayed significant differences in size between the wetter

and drier regions. Fewer leaf lobes and decreased leaf area occurred within the

drier region, and fewer leaves were found on plants within the drier Mustang

population compared to the other three populations. These results suggest that

vegetative organs responded to the limited water resources within the drier

region of Coe by growing smaller and fewer leaves. Limited cell expansion, and

consequent reduction in leaf growth, is one of the primary responses in plants to

drought conditions (Chaves et al, 2003). This reduced growth decreases foliar

surface area, thereby limiting the number of stomatal openings and, as a result,

reduces water loss through transpiration. The reduction in available CO_2

resulting from stomatal closure in dry environments can also contribute to a

reduction in photosynthesis and leaf growth (Chaves et al, 2003). A reduction in

leaf size has been exhibited in a number of studies where plants grew under dry

conditions (e.g., Dudley, 1996; Cordell et al, 1998; Lambrecht, 2010; Ward et al,

2012).

Plasticity in floral and leaf traits

Variation in environmental moisture not only affected floral and leaf sizes but the phenotypic correlations between characters as well. The character correlations analyzed in this study demonstrated how the relationships between characters changed in response to different levels of water availability. Within the wetter and drier regions floral characters were positively correlated with each other, and leaf characters were positively correlated with each other. Within the drier region, more floral character correlations were found than in the wetter region, suggesting that there was more independent character growth within the wetter environment. The increased number of trait correlations within the drier environment may be due to constraints on floral character growth when plants are under environmental stress. Character correlations can result from plastic responses to environmental conditions (e.g. water availability), genetic structure, or a combination of plasticity and genetic framework (Schlichting, 1989; Schlichting and Pigliucci, 1998). For example, in a study of fireweed (*Epolobium angustifolium*, Onagraceae; Carroll et al, 2001), variation in water supply was found to affect character correlations. Flower size and nectar volume were tightly correlated in the drought treatment, while leaf water potential and nectar concentration were correlated only within the controls. Floral traits displayed a less plastic response to drought than did physiological leaf traits.

Results from the *E. angustifoium* study and the current study suggest that trait responsiveness to environmental forces is an important factor in the coupling or uncoupling of characters. Foliar organs control transpiration, carbon fixation, and photosynthesis, and the plasticity displayed in leaf morphology is central to a plant's ability to respond to decreased water supply by controlling these physiological functions. Flowers lack control of these functions; therefore plasticity of floral traits in water-limited environments is reduced. In the current study vegetative traits were not significantly correlated with floral traits, showing a tendency for floral and vegetative characters to be uncoupled along the precipitation gradient. Herrera (2005), and Chalcoff et al (2008), while assessing morphology along environmental gradients, both found that floral traits were correlated within floral organs, and vegetative traits were correlated within vegetative organs, yet floral and vegetative traits were not highly correlated with one another. The uncoupling of floral and leaf traits suggests that leaf characters need to be able to respond to changes in the environment separate from floral trait growth.

Strategies for reducing exposure to water-limited conditions

The decreased character growth displayed in this study may also be a drought escape strategy utilized by plants growing in the drier eastern region of Coe. Plants can effectively escape the driest conditions of the season by quickly growing and reproducing during the earlier part of the season (Galen, 1999;

Heschel and Riginos, 2005; Sherrard and Maherali, 2006; Franks et al, 2007).

Rapid development, and associated decreased plant and floral growth, allows a

plant to reduce the amount of time that it is exposed to dry conditions. Plants

employing this drought escape strategy of rapid development, early flowering,

and reduced floral size are often associated with a transition to self-fertilization

(Mazer et al, 2004; Strauss and Whittall, 2006; Sicard and Lenhard, 2011).

Although no significant difference was found in stigma-to-anther distance

between the wetter and drier regions of Coe, a decrease in herkogamy can be

related to a shift to self-fertilization (Elle and Hare, 2002).

Drought avoidance may also be employed by plants in drier environments

through an increase in WUE. Higher levels of carbon fixed by photosynthesis

compared to water lost through transpiration are associated with higher levels of

WUE in plants. Increased levels of WUE have been detected in plants growing

in drier conditions that displayed a reduction in growth (Sandquist and Ehleringer,

2003; Caruso, 2006; Picotte et al, 2007; Ivey and Carr, 2012; Martin and

Thorstenson, 1988; Dudley, 1996; Caruso et al, 2005). Although floral size and

leaf area both decreased as precipitation declined in the current study, the ratio

of floral area to leaf area (FA/LA) increased from the wetter to the drier region.

Leaves balance internal water reserves by closing their stomata to reduce water

loss, thereby supporting water intensive floral growth. The relationship between

floral size and WUE has been shown through measurements of $\delta^{13}C$ and FA/LA

ratios. An increase in FA/LA has been found to be positively associated with

increased WUE in species of *Achillea* and *Chrysanthemum* (Lambrecht and Dawson, 2007), and in *Leptosiphon bicolor* (Lambrecht, In press). Flowers need water to grow and function, but they are not able to limit water loss through transpiration, and must rely on vegetative structures for their water supply (Galen, 2000; de la Barrera and Nobel, 2004; Galen, 2005; Feild et al, 2009). Increased floral surface area results in greater evaporative water loss from flowers and the higher ratio of floral area to foliar surface area results in leaf structures having to work harder to maintain the plant's water balance. Studies have shown vegetative structures incurring a physiological cost for water lost through floral organs (Galen, 1999; Galen et al, 1999; McDowell and Turner, 2002; Lambrecht and Dawson, 2007).

For the current study, based on $\delta^{13}C$ ratios, no significant relationship between floral size and WUE was detected within the drier or wetter environments. Even though there was no evidence of increased WUE related to the wetter or drier regions for this study, differences in WUE have been detected for *L. androsaceus* during previous years at Coe (Lambrecht, 2010). Lambrecht identified a significant positive relationship between flower size and $\delta^{13}C$ within the drier environment in years of low precipitation. That is, as floral area increased, integrated WUE also increased. This physiological response suggests that as floral area increased, thereby increasing floral transpiration, leaves were forced to reduce water loss by closing their stomata, which increased the plant's WUE.

Given that an increase in WUE associated with flower size in the drier region of Coe was displayed for *L. androsaceus* in past years, perhaps a significant difference in $\delta^{13}C$, as it related to floral size, was not detected between the two regions for this study year because 2011 was an above average rainfall year. Comparing the average rainfall for previous years (2007 through 2010) with rainfall for the year of this study (2011), from the Santa Clara Valley Water District historic precipitation gauge report (ALERT Precipitation Gauge Information, 2012), showed a 22% increase in precipitation within the wetter and drier regions of Coe. Given the relatively high rainfall average for 2011 it is not surprising that a difference in WUE was not detected between plants growing within the wetter and drier environments. Plants growing within the drier region were not sufficiently water-limited to induce a physiological stress response leading to increased foliar stomatal closure. Although the FA/LA ratio was greater within the drier region, floral water costs, and the resultant affect on foliar physiology, were possibly tempered by increased water availability within the drier environment at Coe.

Conclusions

The results from this study exhibited size differences in phenotypic traits of *L. androsaceus* along the precipitation gradient at Henry W. Coe State Park. Floral and vegetative character sizes and numbers had smaller mean values for the drier, eastern region compared to the wetter, western region of Coe. Leaf

and floral characters displayed an acclimation response to the reduction in moisture within the drier region by limiting growth. Studies have shown that maintenance of larger corolla displays are important for pollinator attraction, yet resource diversion to floral growth can be mediated by environmental stress, such as water limitation, thereby reducing corolla size and display (Galen, 1999; Galen, 2000). *Leptosiphon androsaceus* is an insect-pollinated annual, and increased floral display would likely be reproductively advantageous when precipitation levels are high, yet water limitation is conceivably regulating the size of floral growth within the drier region of Coe.

The uncoupling of floral and leaf traits documented in this study suggests that the whole plant benefited from leaf characters responding to environmental forces independent of floral characters. Floral and vegetative characters showed plasticity in size in response to decreased moisture availability within the drier region, yet the lack of floral control of physiological function reduced the need for highly plastic responsiveness in floral traits within the wetter environment. Leaves control transpiration and maintain the water balance for the whole plant; therefore, plasticity in foliar traits is crucial to plant function in drier habitats.

Comparing the two regions, floral area was greater relative to leaf area within the drier environment. Whereas differences in $\delta^{13}C$ ratios with relation to floral size and precipitation were not apparent for this study year, perhaps due to high rainfall for 2011, higher integrated WUE related to flower size within the drier

region was found for Lambrecht's (2010) four year study on the same populations at Coe. Given the higher ratio of FA/LA displayed within the drier region for the current study, increased rainfall for 2011 may have mitigated the water costs that would have been incurred by leaves compensating for larger floral growth within the drier environment.

Natural selection may favor an adaptive strategy of rapid development and decreased plant trait size to avoid drought conditions, and the reduced floral size displayed within the drier region may be due to this strategy of drought escape. Additional study of the temporal phenology of *L. androsaceus* within these populations will further answer the question of whether this species is adapting to drought conditions within the drier region through a drought escape strategy. High genetic variation is thought to be related to drought avoidance strategies in unstable environments and phenotypic and genetic variation within species are essential for adaptation to changing environmental conditions, thereby allowing plants to evolve by natural selection (Sandquist and Ehleringer, 2003). Genetic variation for a range of phenotypic sizes is likely maintained by this species, and it is possible that natural selection is acting on floral and vegetative morphology within the drier region of Coe. A greenhouse study of trait size for this species would be useful to ascertain whether the differences in character size displayed in this study are genetic, or a combination of adaptation and plastic trait responses to environmental fluctuations in precipitation.

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