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Shifts in water availability mediate plant-pollinator interactions

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SUMMARY

- Altered precipitation patterns associated with anthropogenic climate change are expected to have many effects on plants and insect pollinators, but it is unknown if effects on pollination are mediated by changes in water availability. We tested the hypothesis that impacts of climate on plant-pollinator interactions operate through changes in water availability, and specifically that such effects occur through alteration of floral attractants.
- We manipulated water availability in two naturally occurring *Mertensia ciliata* (Boraginaceae) populations using water addition, water reduction, and control plots and measured effects on vegetative and floral traits, pollinator visitation, and seed set.
- While most floral trait values, including corolla size and nectar, increased linearly with increasing water availability, in this bumblebee-pollinated species, pollinator visitation peaked at intermediate water levels. Visitation also peaked at an intermediate corolla length, while its relationship to corolla width varied across sites. Seed set, however, increased linearly with water.
- These results demonstrate the potential for changes in water availability to impact plant-pollinator interactions through pollinator responses to differences in floral attractants, and that the effects of water on pollinator visitation can be non-linear. Plant responses to changes in resource availability may be an important mechanism by which climate change will affect species interactions.

KEY WORDS

Bombus, *Boraginaceae*, climate change, flower size, *Mertensia ciliata*, mutualism, plant-pollinator interactions, precipitation manipulation

INTRODUCTION

Change in water availability is a key mechanism by which climate change will affect plant performance. The direct effects of changes in water availability on plant physiology are well established. Water limitation can reduce the capacity of plants to maintain turgor and transpiration, or to uptake nutrients, and can alter plant-microbial interactions in the soil, all of which can lead to decreased resources for leaves, flowers, fruits, and seeds (Galen et al., 1999; Caruso et al., 2005; Burkle & Irwin, 2009; Berdanier & Klein, 2011; Barber & Soper-Gorden, 2014). Such direct effects on plant physiology may in turn mediate plant interactions with antagonists (Jamieson et al., 2012) and mutualists (Blankinship et al., 2011), including pollinators, but those effects are less well understood (Gornish & Tylianakis, 2013).

Shifts in water availability may have a variety of effects on plant-pollinator mutualisms. One relatively well-studied mechanism is changes in the temporal overlap between flowering and pollinator activity associated with reduced snowpack and early snowmelt (reviewed in Forrest 2015), but it is not the only way changes in water may impact these interactions (Strauss & Whittall, 2006; Gornish & Tylianakis, 2013). Plant responses to differences in water availability may also affect their capacity to attract pollinators via changes in floral attractants. Producing and maintaining flowers is energetically costly, in terms of the initial carbon and nutrients necessary for bud formation (Teixido, 2014), transpirational water loss through inflorescences (Nobel, 1977; Carroll *et al.*, 2001), as well as water diverted from other functions (i.e., photosynthesis) for flower maintenance (Galen *et al.*, 1999). Perhaps because these costs

increase with corolla size (Galen *et al.*, 1999), drought-stressed plants often produce smaller flowers, and may also produce fewer flowers and shorter inflorescences (Mal & Lovett-Doust, 2005; Caruso, 2006; Strauss & Whittall, 2006). In addition, changes in soil moisture can affect the volume and composition of nectar rewards (Zimmerman & Pyke, 1988; Carroll *et al.*, 2001; Nicolson *et al.*, 2007; Burkle & Irwin, 2009; Halpern *et al.*, 2010; Waser & Price, 2016), pollen (Waser & Price, 2016), as well as the emission and composition of floral volatiles (Burkle & Runyon, 2016), all of which may influence the ability of plants to attract pollinators.

Such water-mediated changes in floral attractants may go on to impact pollination through several mechanisms. First, pollinator visitation rates may be impacted by changes in floral display, including plant height and floral abundance. Tall plants and flowers at the tops of inflorescences can receive more pollinator visits, as well as larger and more genetically diverse pollen loads, than their shorter counterparts (Lortie & Aarssen, 1999; Carromero & Hamrick, 2005). As taller inflorescences also tend to have more flowers than shorter stems (Lortie & Aarssen, 1999), pollinator preferences for taller plants may be due, in part, to attraction to plants with more flowers and larger floral displays (Eckhart, 1991; Conner & Rush, 1996; Galen, 2000; Thompson, 2001; Hegland & Totland, 2005; Goulson, 2010). Second, variation in corolla size can affect both pollinator visitation rate (Inouye, 1980; Bell, 1985; Galen & Newport, 1987; Eckhart, 1991; Suzuki, 1994; Conner & Rush, 1996; Galen, 2000; Thompson, 2001) and pollinator effectiveness (Galen & Newport, 1987; Young & Stanton, 1990; Campbell *et al.*, 1991). Third, changes in nectar volume (Inouye, 1978; Pleasants, 1981; Wright, 1988; Real & Rathcke, 1991; Mitchell, 1993; Nicolson *et al.*, 2007; Waser & Price 2016) and composition (Pleasants, 1981; Nicolson *et al.*, 2007; Hoover *et al.*, 2012) can impact visitation rate as pollinators seek the most rewarding options. While many studies have examined either how

water availability influences floral traits or how floral traits influence pollination, evaluating both phenomena in situ provides valuable insight into how climate driven variation influences species interactions.

We investigated the extent to which variation in soil moisture impacts the capacity of plants to attract pollinators and produce seeds. We manipulated water availability during two growing seasons and measured effects on vegetative and floral traits, nectar rewards, pollinator visitation, and plant reproduction. We predicted that plant growth and performance would be influenced by differences in water availability, such that water limitation would (1) reduce floral display (i.e., ramet height and floral abundance), corolla size, and nectar rewards. We also hypothesized that floral responses to reduced soil moisture would (2) negatively impact pollinator visitation rates, which in turn would be (3) associated with a decline in seed set and seed mass.

MATERIALS AND METHODS

Study System

Fieldwork was conducted during the growing seasons of 2013 and 2014 in two subalpine meadows (38°59'48.5"N 107°00'57.5"W, 2,992 m and 39°00'20.3"N 107°01'53.5"W, 3,143 m) located 5.5 and 7.2 km from the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado, USA. Both field sites have similar plant communities, dominated in mid-summer by large populations of the focal species, *Mertensia ciliata* (James ex Torr.) G. Don (Boraginaceae), as well as *Delphinium barbeyi* Huth. and *Veratrum californicum* Durand. Between 1973-2006; mean spring (April-June) temperatures at RMBL have increased by 2.0 °C, and the average date of spring snowmelt has advanced by nearly two weeks (Miller-Rushing & Inouye, 2009). These trends of warmer temperatures and earlier snowmelt, which result in an earlier, longer dry season prior to mid-summer thundershowers (Blankinship *et al.*, 2014), are

expected to continue in the southern Rocky Mountains for the next century (Pederson *et al.*, 2011).

Mertensia ciliata, the tall-fringed bluebell, is an herbaceous perennial found primarily along streams and wet meadows in subalpine and lower alpine zones of the Rocky and Sierra Nevada Mountains. Plants are rhizomatous and form compact clones of a few to several hundred flowering ramets (Pelton, 1961). The pendant, tubular flowers are typically open for six days and can produce a maximum of four one-seeded nutlets. Stigmas are receptive throughout flowering, but pollen is usually removed within 24–48 hours of anther dehiscence by medium and long-tongued bumblebees, including *Bombus balteatus* (Dahlbom), *B. bifarius* (Cresson), *B. flavifrons* (Cresson), and *B. frigidus* (Smith) (Pelton, 1961; Geber, 1985; Suzuki, 1994). Field insect exclusion and hand pollination experiments indicate that, despite being self-compatible, seed set is dependent on insect pollination (Geber, 1985).

In the subalpine meadows around RMBL, *M. ciliata* blooms from late June through late July. Since 1973 *M. ciliata* has become less common at lower elevations ($\leq 2,900$ m), and observed declines in peak floral abundance correlate significantly with earlier snowmelt timing (Miller-Rushing & Inouye, 2009). These patterns suggest that changes in snowmelt timing may be affecting persistence of *M. ciliata* populations.

Experimental Design

To test the hypothesis that plant responses to differences in soil moisture impacts their capacity to attract pollinators and produce seeds, we manipulated water availability in two naturally occurring *M. ciliata* populations using a randomized block design. Within each population (hereafter site), dense patches of *M. ciliata* were marked with flags and designated as blocks. Within each block, three 1 m² treatment plots, separated from the others by 1 m, were

marked with flags and randomly assigned to a precipitation treatment: water reduction, water addition, or ambient. To minimize the flow of resources with ramets outside of the plots, we cut 15 cm into the soil around each plot, bisecting the rhizomatous root system. In 2013, we had five blocks per site ($N_{2013} = 30$) and in 2014 we established new blocks, seven at one site and three at the other ($N_{2014} = 30$).

Precipitation manipulations were maintained through the growing seasons of 2013 (June 18 – August 6) and 2014 (June 21 – August 8) and discontinued at the end of flowering. Water addition treatments received twice the historic average rainfall during July from 1990 to 2009; which amounted to an additional 3.5L of water every 2 d to each 1 m² plot (Campbell & Wendlandt, 2013). We watered plots manually with watering cans slowly and evenly to avoid pooling, in the mid to late afternoon to coincide with the timing of July thundershowers.

Water reduction plots were covered with 1.1 m² rainout shelters designed to intercept 50% of incoming summer precipitation (Yahdjian & Sala, 2002). The larger area covered by the rainout shelters allowed us to minimize edge effects on plants within the 1 m² treatment plots under the shelters. Shelters were built from 1" PVC pipe and slats of U-shaped clear polycarbonate roofing (Palram Americas, Inc., Kutztown, Pennsylvania, USA) that formed a sloping partial roof covering 50% of the top area. The mean height of the shelters was 1.2 m, which was selected to exceed height of the plants in the study area. Slat deposited water into a trough at the down-slope end of the roof, which extended beyond the width of the shelter and shunted the water away from all treatment plots. The open-sided shelters allowed free access to the plants below by pollinators, herbivores, and scientists. Control treatment plots received only natural rainfall. To minimize the visual impact of our research near the Gunnison County road, we spray-painted the rainout shelter frames green and did not install infrastructure control shelters over the water

addition and control treatments. Comparisons of light levels between plots with and without shelters revealed no significant difference (Supporting Information Notes S1).

Throughout the experiment, we measured soil moisture as volumetric water content (VWC) every other day using a 12 cm Campbell Scientific “HydroSense” probe inserted into the center of each plot (always before applying water). Control plots received average precipitation slightly more than the historical average over the past 15 years (88 vs. 84 mm). The treatments led to 9.4%, 11.7%, and 15.4% average VWC in the soil for dry, control, and wet plots, creating a gradient in soil moisture. Average summer (June-July) VWC values for plots were analyzed with ANOVA with the factors of treatment and block. Although we found significant differences among the treatments in both years ($F_{2,48} = 16.55$, $P < 0.0001$), due to large within treatment variation among blocks (39% variance within treatments) we treated soil moisture as a continuous rather than discrete variable in our analyses. We tracked soil VWC of a subset of plots at a finer temporal scale as well (Supporting Information Fig. S1).

Vegetative and floral trait measurements

We marked a randomized subset of ten ramets per plot, for which we measured vegetative traits, corolla size, and flower abundance. Vegetative traits included total leaf count and ramet height as measured from the base to the tip of the most terminal leaves. Vegetative traits were measured on July 16, 2013 and July 9 and 12, 2014, during peak flowering. For 3.7 ± 0.1 (Mean \pm SEM) flowers per ramet on July 11-15, 2013 and July 2-29, 2014, we measured corolla width at the opening of the tube and corolla length from the base of the calyx to a randomly chosen corolla lobe. At the end of each flowering season, we determined the total number of flowers produced by each marked ramet by counting all reproductive structures (buds, aborted buds, flowers, fruits, aborted fruits).

During the second summer, we also counted the total number of ramets per plot, the number that flowered, and total floral abundance at the end of the season. For an additional five randomly selected ramets per plot, in 2014, we measured production of nectar rewards (i.e., nectar volume and concentration). To do this, we excluded pollinators from newly opened flowers using sheer organza draw-string pouches (Uline, Pleasant Prairie, WI, USA). After 48 hours, we measured nectar volume using 5 µl microcapillary tubes (Kearns & Inouye, 1993) and percent sugar concentration using a handheld nectar refractometer (Bellingham + Stanley Ltd., Basingstoke, Hants, UK). Nectar measurements were based on an average of 3.2 ± 0.2 flowers per ramet collected between July 7-29, 2014.

We analyzed the effects of experimental variation in soil moisture on vegetative and floral traits using the plot mean of each trait, calculated from the individual ramet means when appropriate, as the response in linear models with main effects of mean summer VWC and site as predictors. For those traits with two years of data, we included main effects of year, as well as mean summer VWC \times year and site \times year interactions as predictors. When interactions were not significant, we reran the models without the interaction to simplify interpretation. In these and all other analyses, mean summer VWC \times year was not significant and so was excluded. To see if the observed differences in corolla size were explained by a general increase in ramet size, we performed an additional analysis with mean ramet height added as a model covariate.

Residuals of vegetative, corolla, and nectar traits were approximately normally distributed (Shapiro-Wilk, $P > 0.05$). We removed one outlier from our analysis of peak floral abundance that fell 30 standard deviations away from the mean, a significant deviation (Grubbs test, $P = 0.0001$, R package *outliers*, Lukasz, 2011). After removing this outlier, the residuals of all floral abundance measurements were also normally distributed (Shapiro-Wilk, $P > 0.05$). All analyses

were conducted using R (R Core Team, 2015). For models including interaction terms we calculated type III sums of squares using the *car* package in R (Fox & Weisberg, 2011) and with a contrast setting that sets the row sum to zero. For models without interaction terms, we report type II analyses. Block was not found to be a significant predictor in any of our analyses and so was removed as a factor for simplicity.

Pollinator visitation measurements

To test the hypothesis that floral responses to differences in water availability impacts pollinator visitation, we conducted pollinator observations at the whole plot level for a total of 93.5 hours (18.5 hours in 2013 and 75 hours in 2014). In 2014, we also directly tested the hypothesis that water-mediated changes in corolla size reduces pollinator visitation rates by tracking pollinator visitation to the ten marked ramets in each treatment plot for which we had specific data on corolla traits. We tracked pollinator identity and number of flowers visited during 30-minute observation periods between the hours of 9:00 and 16:00, when weather conditions allowed pollinator activity, between July 9-10, 2013 and July 2-31, 2014. Observations in 2013 occurred during peak flowering, while those in 2014 covered the whole flowering period for most of the plants in both communities. We observed all plots within a block on the same day, and on all blocks before repeating the cycle. The order of observations was randomized among blocks and among treatment plots within blocks. Visitors were determined to be pollinators if they crawled inside the flower corolla. Of the 303 pollinators observed, two were flies, eight were solitary bees and the rest were bumblebees. Excluding non-bumblebee visitors from analyses did not alter results, therefore we report results including all visitors. Open flowers within plots and on marked ramets were counted at the beginning of each observation period. We calculated pollinator visitation rate at the whole plot level ($N = 60$) and

for individually marked ramets ($N = 224$) as (total number of flowers visited / number of flowers available per hour of observations), averaged across the flowering season.

Pollinator responses to experimental variation in soil moisture were tested in a model using mean pollinator visitation rate per plot as the response. Predictors included mean summer VWC, site, year, site \times year interaction, as well as a quadratic term for mean summer VWC, to test for a curvilinear relationship between visitation and soil moisture. We assessed whether changes in nectar availability influenced mean pollinator visitation rate per plot in a model with linear and quadratic terms for mean summer VWC, site, mean nectar volume, and site \times nectar volume interaction as predictors (2014 data only, $N=30$). We tested whether pollinators respond to water-mediated changes in corolla size using mean pollinator visitation rate to individual ramets as the response variable. For each trait (corolla length or corolla width), we first tested whether selection varied by site, using a model with linear and quadratic terms for the trait, site, and the site by linear and quadratic terms as predictors. As we detected significant site by trait interactions, we ran final models separately by site, with linear and quadratic terms for the trait as predictors.

The residuals of these pollination analyses were not normally distributed (Shapiro-Wilk, $P \leq 0.05$), likely due to a high proportion of observation periods with zero pollinator visits. For this reason, we employed a zero-inflated mixture model with the package *pscl* in R (Jackman, 2008), which modeled the pollinator visitation rate with a Poisson distribution and the excess zeroes with a binomial distribution and logit link. In each case, the zero-inflated model provided a better fit than a GLM with Poisson distribution, as judged by a lower value for Akaike Information Criterion (AIC).

To quantify responses of pollinator visitation to individual traits in a way comparable to other

studies, we used phenotypic selection analysis with pollinator visitation rate to individual ramets as the fitness measure (Lande & Arnold, 1983). Pollinator visitation rate for each ramet was divided by the mean to estimate an index of relative fitness. For each corolla trait and site, we calculated standardized directional (S') and quadratic (γ) selection differentials from linear regression models of relative fitness on standardized trait values (mean of 0, variance of 1). To estimate the strength of stabilizing selection, we calculated quadratic selection differentials (γ) by including both trait and trait² as predictors in the regression model, and doubling the quadratic regression coefficients (Stinchcombe *et al.*, 2008). We also tested whether selection varied with water availability by examining relative fitness as a function of the standardized trait value, water treatment and the trait \times water treatment interaction. In cases where we found a significant interaction, we estimated directional (S') and quadratic (γ) selection differentials separately for each corolla trait and water treatment (i.e., water reduction, control, and water addition).

Seed set measurements

We counted the total number of nutlets produced per flower (measured as described by Forrest & Thompson, 2010). Because *M. ciliata* can produce a maximum of four nutlets per flower, we calculated seed set as $(100\% \times \text{number of mature nutlets}) / (4 \times \text{number of flowers})$. Mature nutlets from each marked ramet were collected in coin envelopes and transported to the University of California, Irvine where they were weighed. We calculated mean seed mass for a ramet as $(\text{mass of collected nutlets} / \text{number of collected nutlets})$. We analyzed the effects of experimental variation in soil moisture on mean seed set and mean seed mass for each plot as we did with vegetative and floral traits. Neither fitness component showed a significant quadratic relationship with soil moisture, so we removed that term from both analyses.

We used a GLM model with Poisson distribution for the seed set analysis, as the residuals

were not normally distributed (Shapiro-Wilk, $P \leq 0.05$) and it provided a better fit than other models as judged by a lower AIC value. We used a normal distribution for seed mass (Shapiro-Wilk, $P > 0.05$). To assess whether differences in seed set are associated with pollinator visitation rate, we performed an additional analysis including linear and quadratic terms for mean pollinator visitation rate at the plot level as a factor in the model along with mean summer VWC, site, and year. We also estimated directional and stabilizing selection on corolla width and length using phenotypic selection analysis, as described above, except with seed set as the fitness measure.

RESULTS

Effects on vegetative and floral traits

Prediction 1: Water-limitation will reduce floral display, corolla size, and nectar rewards. As predicted, corolla width increased with mean soil moisture (June-July) ($F_{1,54} = 37.09$, $P < 0.0001$, Fig. 1a), as did corolla length ($F_{1,55} = 16.45$ in a model with non-significant site \times year interaction removed, $P = 0.0002$, Fig. 1b). When we added mean ramet height to these models, corolla width and length still increased significantly with soil moisture, indicating the responses of floral morphology were not due simply to an increase in plant size. Nectar volume also increased with soil moisture ($F_{1,27} = 5.61$, $P = 0.025$, Fig. 1c), although nectar concentration did not ($F_{1,27} = 0.23$, $P = 0.63$). We saw the same patterns for floral traits when looking at June and July soil moistures separately, except for nectar volume which responded significantly to water in July ($F_{1,27} = 8.96$, $P = 0.006$) but not June ($F_{1,27} = 0.02$, $P = 0.9$).

Most measures of floral abundance that we tracked, including floral abundance of flowering plants and proportion of flowering plants per plot, were not influenced by changes in water availability ($P > 0.05$, Supporting Information Table S1). Peak floral abundance varied with soil

moisture, but the pattern differed significantly between the two sites ($F_{1,25} = 4.71$, $P = 0.04$). At the higher elevation site, peak floral abundance increased with soil moisture, whereas it decreased with soil moisture at the lower elevation site (Supporting Information Fig. S2a). Leaf count did not vary with changes in water availability ($F_{1,52} = 0.04$ in a model with non-significant site \times year interaction removed, $P = 0.8$), but ramet height increased with soil moisture ($F_{1,52} = 4.78$, $P = 0.03$, Supporting Information Fig. S2b).

Effects on pollinator visitation

Prediction 2: Floral responses to reduced soil moisture will negatively impact pollinator visitation rates. The probability of a plot receiving no pollinator visits in an hour of observations did not vary significantly with any predictor (zero-inflation part of the model, Table 1). Among those plots that received visits, pollinator visitation rates were highest for plots with intermediate soil moistures (Fig. 2a), yielding a quadratic as well as a linear effect for the count part of the model (Table 1). There was also a significant site \times year interaction, with higher mean visitation rates in 2013 than 2014, and a slightly bigger difference at the low site (count part of the model, Table 1). Pollinator visitation at the plot level in 2014 was not influenced by changes in nectar volume ($Z_{\text{zero-inflation model}} = -0.66$, $Z_{\text{count model}} = 1.71$, $DF = 1, 28$, $P = 0.09$).

At the individual ramet level, the likelihood that a pollinator would visit a plant did not vary with corolla width or length (zero-inflation part of the model, Table 2). When pollinators did visit, however, plants at the low site with intermediate corolla sizes and plants at the high site with intermediate corolla lengths received more visits per flower than those with smaller or larger corollas (Fig. 3a,b,d), yielding a quadratic as well as a linear effect for the count part of the model (Table 2). The standardized selection differentials and the quadratic selection differentials indicate that corolla length, and width at the low site, is subject to pollinator-mediated positive

directional selection for larger corollas, as well as stabilizing selection for intermediate corollas (Fig. 3a,b,d). At the high site, however, we found evidence for weak disruptive selection on corolla width, where narrower and wider flowers received more visits per flower than those with intermediate corolla widths (Fig. 3c, Table 2). Selection via pollinator visits differed among water treatments, judging by a significant trait \times water treatment interaction term in the zero-inflation models ($P \leq 0.05$). Selection via pollinator visits shifted from strongly directional for large flowers under water reduction to stabilizing selection under water addition (Table S2).

Effects on seed set

Prediction 3: Reduced pollinator visitation associated with drought-induced changes in floral traits will negatively impact seed set and seed mass. At the plot level, mean seed set increased linearly with soil moisture (likelihood ratio $\chi^2_{1,56} = 14.46$ in Poisson model with non-significant site \times year interaction removed, $P = 0.0001$, Fig. 2b), but mean seed mass did not ($F_{1,55} = 3.6$, $P = 0.06$). When mean pollinator visitation rate was added to this model, we found seed set increased with pollinator visitation, but only up to a point (Linear: $\chi^2_{1,54} = 16.63$, $P < 0.0001$; Quadratic: $\chi^2_{1,54} = 11.17$, $P = 0.0008$, Supporting Information Fig. S3). Soil moisture retained an effect in the model as well. Thus, seed set responded both to pollinator visitation and through other mechanisms to soil moisture. In 2014, the standardized selection differentials for corolla width and corolla length (Table 3) indicated positive directional selection for both traits via seed set at the low site, but no significant selection at the high site. We also tested for stabilizing selection on corolla size through seed set, but it was not significant ($P > 0.05$). When we tested for an interaction between corolla traits and water treatment, we found no evidence that selection based on seed set varied with water treatment ($P > 0.05$).

DISCUSSION

Effects of variation in soil moisture on plant-pollinator interactions

By following responses to experimental variation in soil moisture over two years under natural field conditions, we tested the hypothesis that impacts of climate on plant-pollinator interactions operate through changes in water availability. We found strong evidence for non-linear effects of soil moisture on pollinator visitation, largely mediated through changes in corolla size and floral display size. Although pollination peaked at intermediate soil moistures and corolla lengths, seed set continued to increase with additional moisture, even as visitation declined.

Effects on vegetative and floral traits

Both corolla size and nectar production were strongly influenced by changes in soil moisture. Phenotypic plasticity in these traits, such as we observed (Fig. 1), likely represents a trade-off between dealing with environmental stress and pollinator preference (Strauss & Whittall 2006). In sub-alpine and alpine systems, where growing seasons are short, having the ability to shift resources away from floral attractants may be adaptive for perennial species that have multiple opportunities to reproduce and are presented with inter-annual climate variability (Stinson, 2004). For example, drought-stressed *Polemonium viscosum* that diverted resources from leaves to larger flowers experienced lower fitness, both in terms of seed set and post-reproductive survival the following year, than plants that did not produce large flowers during drought (Galen, 2000). The strength of this trade-off between environment and pollinator preference, however, likely varies among different plant-pollinator systems (Thompson, 2001; Hegland & Totland, 2005) and among populations within plant-pollinator systems (Eckhart, 1991; Caruso, 2006). In contrast to corolla size and nectar volume, changes in soil moisture during the growing

season had little effect on most measures of floral display. Only ramet height and peak floral abundance at the high site increased with water (Supporting Information Fig. S2). If *M. ciliata* preforms buds or uses stored resources to jump-start spring growth, as is common among alpine and subalpine plant species (Billings, 1974), the impact of changes in water availability on floral abundance may only become evident over multiple years.

Effects on pollinator visitation

We found evidence of both directional and stabilizing selection on corolla size based on pollinator visitation rate, except for corolla width at the high site, which experienced disruptive selection. Detecting directional selection for larger flowers is fairly common (reviewed in Harder & Johnson, 2009), perhaps because larger flowers are more conspicuous and often provide larger rewards to pollinators (Hegland & Totland, 2005). Our estimates of directional selection ($S' = 0.03$ to 0.47) were similar to the strength of directional selection seen in other studies of flower size (Harder & Johnson, 2009). Reports of stabilizing and disruptive selection are less common, in part because they are less often tested for. In *Mertensia ciliata*, stabilizing selection on corolla length may arise in part from the behavior of specific bumblebee species. Both nectar- and pollen-foraging worker bumblebees can assort themselves among plant species by body size (Peat *et al.*, 2005; Corbet & Huang, 2014). The range of potential bumblebee species and castes that can access rewards in flowers may be restricted when corollas are too long (Inouye, 1980; Suzuki, 1994). But, visitation rates by longer-tongued bees may also decline when corollas are too short (Peat *et al.*, 2005), resulting in stabilizing selection with a broad fitness peak. The switch from directional selection for larger flowers under water reduction to stabilizing selection under water addition may simply reflect an overall optimal size for foraging by the bumblebee species that visit *M. ciliata*. It is also theoretically possible that the relationship of pollinator

visitation to flower size resulted in part from the influence of volatiles or other unmeasured traits.

While pollinators showed a consistent preference for intermediate corolla lengths, pollinators responded differently to changes in corolla width at the two sites. At the low site, pollinator visitation peaked with intermediate corolla widths, but at the high site pollinators exhibited disruptive selection on corolla width, with wider and narrower flowers receiving more visits than flowers of intermediate widths. As with corolla length, bumblebees may preferentially visit flowers with corolla widths that match to their body size, as these may be more accessible (Suzuki, 1994) and allow for a faster handling time (Inouye 1980; Peat et al., 2005). It is unclear, however, why pollinators would show a preference for intermediate flowers at the low site and not at the high site. Not only do the two sites share similar plant communities, but the *M. ciliata* populations at both sites were visited by the same assemblage of pollinator species, largely dominated by *Bombus flavifrons* and *B. frigidus*. Even though the same species are present at both sites, it is conceivable that there were differences in the size distribution of visitors to *M. ciliata*.

In addition to trait-mediated pollinator responses to individual ramets, changes in community-level interactions in and around the plots may also explain why pollinator visitation peaked at intermediate water levels. At the community-level, pollinator visitation may vary according to the spatial distribution of conspecifics, as well as the spatial distribution of and rewards offered by other co-flowering species (Goulson, 2010). Moreover, as floral abundance increases, at either the plant or community-level, the proportion of open flowers that are visited per plant may decline (Geber, 1985; Harder & Barret, 1995). If co-flowering species abundance was higher in blocks with high soil moisture, and those species compete for pollinator visits, some of the decline in pollinator visitation at high soil moistures may have been due to potentially larger

floral displays or rewards from co-flowering species in and around the plots.

Larger nectar rewards from *M. ciliata* flowers, however, did not influence pollinator visitation. There are at least two possible reasons why pollinators did not respond to increased nectar availability. First, pollinators may use more obvious visual cues, such as flower size, as a proxy for the size of the floral reward (Hegland & Totland, 2005). Second, while pollinators certainly visit *M. ciliata* to consume nectar, pollen may be the primary resource that attracts some pollinators to *M. ciliata* flowers. If water availability affects pollen quantity or quality, as has been suggested in other studies (Turner, 1993; Waser & Price, 2016), then this might influence the behavior of pollen-collecting visitors, including bumblebees (Harder, 1990).

This experiment showed that changes in water availability can impact plant-pollinator interactions, and that the effects of water on pollinator visitation can be non-linear, but one important caveat is in order. Our data showed that pollinators preferred intermediate length flowers when a range of sizes were available, however, we cannot know whether this preference would be maintained if an entire population were to undergo a drought or receive abundant rain. It is possible that if all plants in the population experienced a drought and produced short flowers, pollinators would avoid *M. ciliata* altogether. But it is also possible that under such conditions, pollinators would visit *M. ciliata* despite the smaller flower size when not given a choice.

Effects on seed set

Seed set increased linearly with the higher water availability expected in later snowmelt years (Fig. 2b), and that increase is likely explained both by pollinator visitation and by other mechanisms. Indeed, in a model including both terms, water and pollinator visitation rate independently influenced seed set. Pollinator visitation, however, declined at high soil moistures

(Fig. 2a), while seed set continued to increase with the additional resources. This result suggests that, while some minimum level of pollinator visitation is necessary for *M. ciliata* to set a full complement of seed (Geber, 1985, Supporting Information Fig. S3), seed set may not have been pollinator limited at most soil moisture levels. In attempts to estimate pollen limitation with pollen supplementation experiments, hand-pollinated plants made fewer seeds than insect-pollinated plants (Gallagher, unpublished data). This result is not easily interpreted as it can be due to a variety of mechanisms including crowding of pollen tubes, stigma damage, or missing peak receptivity (Young & Young, 1992).

The observed increase in seed set may be due to a general expansion in carbohydrate availability for growth and easier acquisition of nutrients when water is abundant, which is also apparent from the increase in ramet height at higher soil moistures (Supporting Information Fig. S2b). Since seed set increased linearly with water availability, as did corolla size, the result was net directional selection on corolla size when measured by seed set at the low site.

Conclusions and lessons for studies of climate change

We found that changes in water availability during the growing season can affect the ability of plants to attract pollinators, as well as their seed production. While the water manipulation methods we used do not capture the effect of altered snowmelt on growing season length, they do capture the changes in soil moisture that accompanies altered snowmelt. In these subalpine communities, accelerated timing of snowmelt with climate change is predicted to increase the overall length of the growing season and the length of the dry period experienced prior to the onset of mid-summer thunderstorms (Pederson *et al.*, 2011), with effects on soil moisture that can last for months (Blankinship *et al.*, 2014).

Pollinator visitation to *Mertensia ciliata* peaked at intermediate soil moistures, which suggests

that it may be important to consider whether changes in the variance, as well as mean, water availability over multiple years impacts plant-pollinator interactions. Around RMBL, the average snowmelt date is $< 1\%$ earlier than it was a decade ago, but year-to-year variation in snowmelt date has increased by 20% (Barr, 2016). These swings in snowmelt date translate into large swings in soil moisture during the growing seasons (Blankinship *et al.*, 2014) and may have important implications for plant-pollinator interactions.

We had expected that increasing water would lead to increased floral display, corolla size and nectar rewards, and these in turn would translate into increased pollinator visitation and seed set. But that is not what we found. Pollinator visitation declined both when water was limited *and* when water was super abundant, but seed set increased monotonically with additional water. This decoupling of pollinator visitation from seed set at higher soil moistures suggests that for *M. ciliata*, seed set responds to other factors, in addition to some minimum level of pollinator visitation necessary to achieve seed set. Understanding the demographic consequences of altered precipitation patterns associated with anthropogenic climate change, therefore, will require determining how those factors that influence seed set respond to changes in water availability over time.

In our system, the non-linear effects of water on pollinator visitation were mediated by responses of bumblebees to differences in corolla length. We may expect to see similar non-linear effects of water on pollinator visitation in systems where flower size has high phenotypic plasticity and where pollinators are known to respond to differences in flower size, as in bee, hummingbird, hawkmoth, and butterfly pollinated species (e.g., Campbell *et al.*, 1991; Eckhart, 1991; Thompson, 2001). As the frequency of extreme summer climates increases (Pederson *et al.*, 2011), plants that experience selection for intermediate floral traits may be between the

proverbial rock and a hard place. If both too little and too much water can drive plants to produce floral displays that are less attractive or less accessible to their current pollinators, over time the accumulation of low-pollination years may affect the persistence of these populations. Testing the net effect that increased interannual variation in water availability has on plant-pollinator interactions and, potentially, on plant fitness, will be an important area of future research.

Shifts in abiotic conditions due to climate change may have important consequences for plant-pollinator mutualisms. Much of the focus in pollination biology as it relates to climate change has been on documenting patterns of phenological variation and modeling the potential consequences of changes in temporal overlap between flowering and pollinator activity (reviewed in Forrest, 2015). This study demonstrates that, even without changes in phenology, climate change may affect plant-pollinator interactions through responses of floral traits to changes in water availability. Only by considering and comparing each of these potential climate impacts can we predict the overall consequences of global climate change.

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AUTHOR CONTRIBUTIONS

M.K.G. and D.R.C. designed the research, conducted fieldwork, analyzed the data and wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Notes S1 Comparison of light levels between plots with and without shelters.

Fig. S1 Mean daily maximum soil volumetric moisture content for (a) 2013 and (b) 2014.

Fig. S2 Effects of variation in soil moisture on *Mertensia ciliata* (a) peak floral abundance and (b) ramet height at the high elevation site (3,143 m.) and low elevation site (2,992 m.).

Fig. S3 Effects of pollinator visitation rate per plot on mean seed set of *Mertensia ciliata* (N = 60).

Table S1 Effects of water availability on floral abundance measurements.

Table S2 Standardized directional (S') and quadratic (γ) selection differentials of pollinator visitation on *Mertensia ciliata* corolla width and length, estimated separately for each corolla trait and water treatment.

TABLES

Table 1 Results of zero-inflation model analysis testing effects of experimental variation in soil moisture on *Mertensia ciliata* pollinator visitation rate per plot.

Count model (Poisson with log link)			
Variable	Estimates	Z value	P
Soil moisture	0.18	3.76	0.0001
Soil moisture (quadratic)	- 0.01	- 3.26	0.001
Site	- 0.09	- 2.78	0.005
Year	0.37	9.71	< 0.0001
Site × Year	- 0.07	- 2.04	0.04
Zero-Inflation model (binomial)			
Variable	Estimates	Z value	P
Soil moisture	- 0.61	- 1.33	0.18
Soil moisture (quadratic)	0.03	1.29	0.19
Site	- 4.07	- 0.005	0.99
Year	4.72	0.005	0.99
Site × Year	3.55	0.004	0.99

Mean pollinator visitation rate per plot, calculated as (total number of flowers visited / number of flowers available per hour of observations) averaged for each flowering season, was used as the response in model selection with linear and quadratic terms for mean summer volumetric water content (VWC), site, year, and site × year interaction as predictors (N = 60).

Table 2 Results for zero-inflation model analysis testing responses of pollinator visitation to differences in *Mertensia ciliata* corolla width and length at two sites.

Site	Variable	Count portion of model			Zero-Inflation portion		
		(Poisson with log link)			(binomial with logit link)		
		Estimate	Z value	P	Estimate	Z value	P
High	Corolla width	- 2.27	- 4.27	< 0.0001	1.51	0.42	0.67
High	Corolla width (quadratic)	0.18	3.95	< 0.0001	- 0.18	- 0.59	0.56
Low	Corolla width	1.10	3.83	< 0.0001	- 1.32	- 0.58	0.56
Low	Corolla width (quadratic)	- 0.09	- 3.71	< 0.0001	0.08	0.38	0.70
Site	Variable	Count portion of model			Zero-Inflation portion		
		(Poisson with log link)			(binomial with logit link)		
		Estimate	Z value	P	Estimate	Z value	P
High	Corolla length	3.51	5.13	< 0.0001	1.49	0.45	0.65
High	Corolla length (quadratic)	- 0.13	- 5.44	< 0.0001	- 0.06	- 0.55	0.59
Low	Corolla length	6.85	8.28	< 0.0001	- 2.6	- 0.77	0.44
Low	Corolla length (quadratic)	- 0.22	- 8.26	< 0.0001	0.08	0.66	0.51

Mean pollinator visitation rate to individual ramets, calculated as (total number of flowers visited) / (number of flowers available per hour of observations) averaged across the flowering season, was used as the response, with either corolla width or corolla length, as well as the quadratic term for that corolla trait as predictors ($N_{\text{high}} = 86$, $N_{\text{low}} = 138$). Estimates based on multiplying visitation rate by 100 and converting it to an integer.

Table 3. Standardized directional (S') selection differentials on *Mertensia ciliata* corolla width and length, estimated separately for each site, using relative seed set as the measure of fitness and standardized linear and quadratic corolla trait values in the model.

	Corolla width			Corolla length		
	S'	χ^2	P	S'	χ^2	P
High site	0.11	1.65	0.20	0.07	1.31	0.25
Low site	0.15	5.63	0.02	0.13	4.16	0.04

Significance estimates based on likelihood ratio test for Poisson models ($N_{\text{high}} = 86$, $N_{\text{low}} = 138$).

FIGURE LEGENDS

Fig. 1 Effects of experimental variation in soil moisture on (a) corolla width, (b) corolla length, and (c) nectar volume of *Mertensia ciliata*. Corolla traits were measured in both 2013 and 2014 (N = 60), whereas nectar measurements were only collected in 2014 (N = 30).

Fig. 2 Effects of experimental variation in soil moisture on *Mertensia ciliata* (a) pollinator visitation rate per plot, calculated as (total number of flowers visited / number of flowers available per hour of observations) averaged across the flowering season and (b) mean seed set per plot, calculated as $(100\% \times \text{number of mature nutlets}) / (4 \times \text{number of flowers})$ (N = 60). Fit line for pollinator visitation was plotted with coefficients from the count portion of the zero-inflated Poisson model from Table 1, using the inverse log link to obtain expected values and shows the relationship with water only.

Fig. 3 Effects of variation in flower size in *Mertensia ciliata* on pollinator visitation rate of individual ramets, calculated as (total number of flowers visited / number of flowers available per hour of observations) averaged across the flowering season (N = 224). (a) corolla length at high site; (b) corolla length at low site; (c) corolla width at high site; (d) corolla width at low site. White square: Water reduction. Black circle: Control. Gray triangle: Water addition. Functions plotted are the fits from the count portion of the zero-inflated Poisson model, using the inverse log link to obtain expected values. In all cases both the linear and quadratic terms were significantly different from zero in the count portion of the model (all $P < 0.0001$), but not in the zero-inflation portion (all $P > 0.4$). S' = standardized linear selection differential. γ = standardized quadratic selection differential.

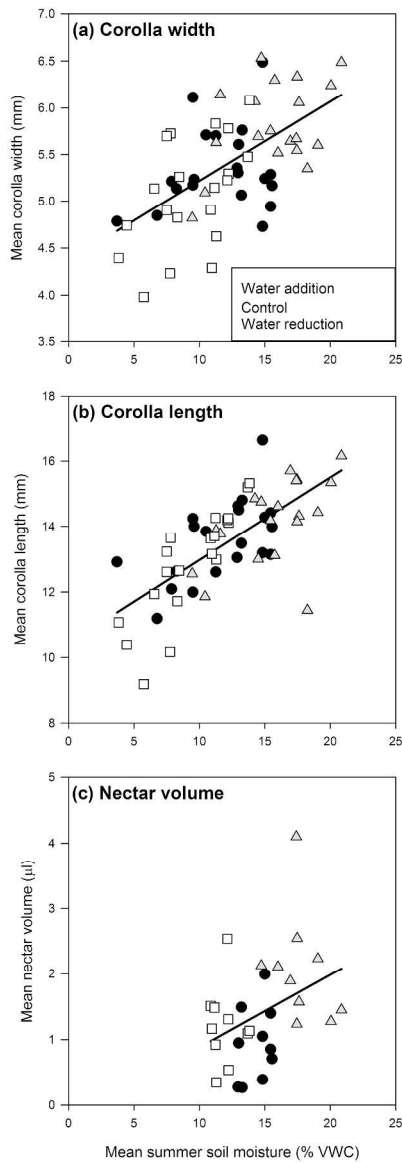


Fig. 1 Effects of experimental variation in soil moisture on (a) corolla width, (b) corolla length, and (c) nectar volume of *Mertensia ciliata*. Corolla traits were measured in both 2013 and 2014 (N = 60), whereas nectar measurements were only collected in 2014 (N = 30).

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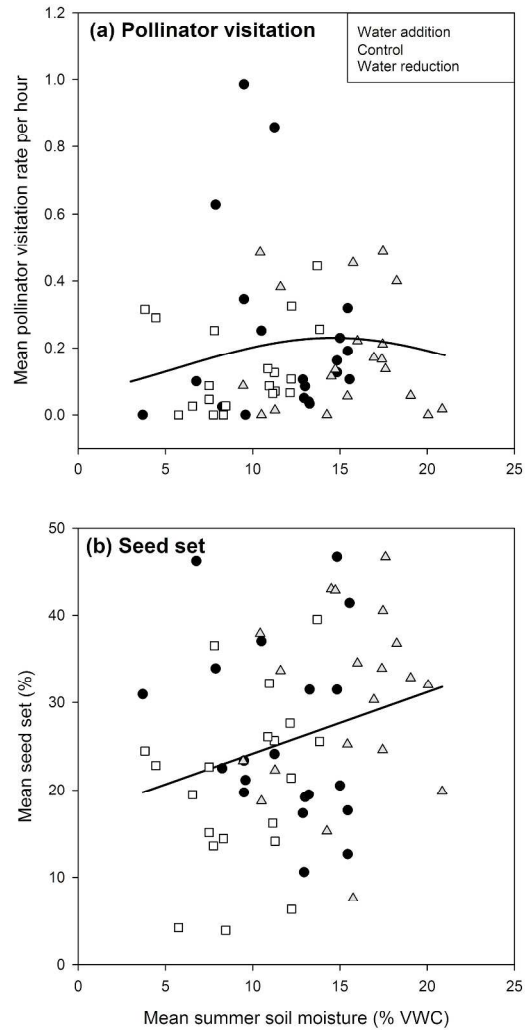


Fig. 2 Effects of experimental variation in soil moisture on *Mertensia ciliata* (a) pollinator visitation rate per plot, calculated as (total number of flowers visited / number of flowers available per hour of observations) averaged across the flowering season and (b) mean seed set per plot, calculated as $(100\% \times \text{number of mature nutlets}) / (4 \times \text{number of flowers})$ ($N = 60$). Fit line for pollinator visitation was plotted with coefficients from the count portion of the zero-inflated Poisson model from Table 1, using the inverse log link to obtain expected values and shows the relationship with water only.

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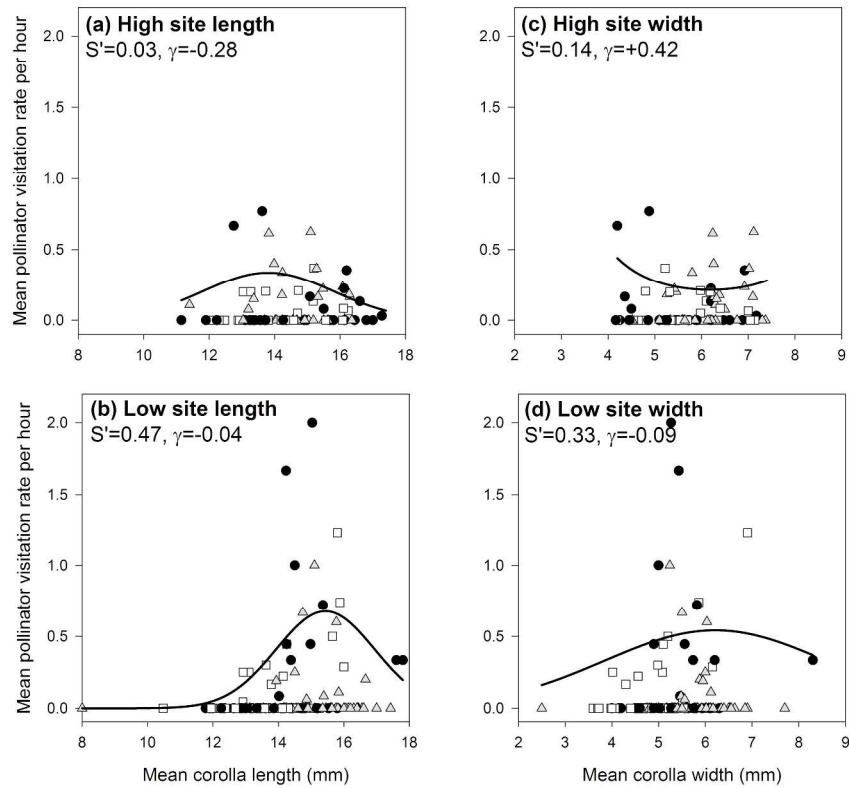


Fig. 3 Effects of variation in flower size in *Mertensia ciliata* on pollinator visitation rate of individual ramets, calculated as (total number of flowers visited / number of flowers available per hour of observations) averaged across the flowering season (N = 224). (a) corolla length at high site; (b) corolla length at low site; (c) corolla width at high site; (d) corolla width at low site. White square: Water reduction. Black circle: Control. Gray triangle: Water addition. Functions plotted are the fits from the count portion of the zero-inflated Poisson model, using the inverse log link to obtain expected values. In all cases both the linear and quadratic terms were significantly different from zero in the count portion of the model (all $P < 0.0001$), but not in the zero-inflation portion (all $P > 0.4$). S' = standardized linear selection differential. γ = standardized quadratic selection differential.