

**ALTERED PRECIPITATION AFFECTS PLANT HYBRIDS DIFFERENTLY
THAN THEIR PARENTAL SPECIES¹**

DIANE R. CAMPBELL^{2,3,4} AND CAMILLE WENDLANDT³

²Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697 USA; and

³Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, Colorado 81224 USA

- *Premise of the study:* Future changes in environmental conditions may alter evolutionary processes, including hybridization in nature. Frequency of hybrids could be altered via range shifts by the parental species or by changes in prezygotic or postzygotic reproductive isolation. We examined the potential for range shifts and change in postzygotic isolation by determining effects of increasing and decreasing precipitation on leaf physiology and fitness components in the subalpine herbs *Ipomopsis aggregata* (Polemoniaceae), *I. tenuituba*, and their natural hybrids in a common garden in the habitat of *I. aggregata*.
- *Methods:* Summer precipitation was experimentally doubled or halved over 3 yr in comparison with ambient conditions. We measured relative growth rate, specific leaf area, intrinsic water-use efficiency, survival to reproduction, biomass, number of flowers produced, and floral morphology.
- *Key results:* *Ipomopsis tenuituba* increased relative growth rate with higher precipitation more so than did *I. aggregata* during the first summer, but this response did not result in changes across treatments in relative survival or final reproductive success of the two species. When precipitation was reduced, the relative success of hybrids was greater than that of the home species, *I. aggregata*. In dry conditions, hybrids increased water-use efficiency and fitness as indexed by number of flowers more so than the other plant types did.
- *Conclusions:* Increased reproduction in hybrids in the reduced precipitation regime indicates that postzygotic reproductive isolation may breakdown under imposition of dry conditions. These results suggest the potential for frequency of hybrids to increase if severe droughts become more common.

Key words: hybrid; hybrid zone; *Ipomopsis*; Polemoniaceae; precipitation manipulation; water-use efficiency.

One of the challenges in predicting how species abundances and distribution will respond to climate change is the potential for shifts in evolution in response to changes in key environmental variables (Hoffmann and Sgro, 2011). Evolutionary adaptation to a new regime of natural selection might occur fast enough to prevent local extinction, although incorporation of such evolutionary responses into predictions of range shifts is still in its infancy (Bridle and Vines, 2007; Hoffmann and Sgro, 2011; Banta et al., 2012). The level of hybridization and introgression between related species is another form of potential evolutionary response to global change that has only recently been explored (Garroway et al., 2010).

Hybridization is common in flowering plants, occurring in 16% of vascular plant genera (Whitney et al., 2010a). Hybridization is also a critical evolutionary process because it represents either ongoing speciation or the breakdown of reproductive isolation in secondary contact. It can introduce new genetic variation into a population (Hewitt, 1988) that allows further adaptation to novel abiotic conditions, as seen in sunflowers (Rieseberg et al., 2007; Whitney et al., 2010b). The frequency of successful hybrids seen in nature could be impacted in multiple ways by changes in environmental conditions. For

example, climate change might alter the ranges of the two related species such that the degree of spatial overlap, and hence opportunity for hybridization, is either reduced or increased (Fig. 1A; Garroway et al., 2010). This effect on range overlap would correspond to a change in reproductive isolation due to increased, or reduced, viability of an immigrant species when arriving in a new habitat (Nosil et al., 2005). Second, a change in environmental conditions in an area of current overlap between two species may increase or decrease the rate at which hybrids are formed (Fig. 1B), for example, by changing the similarity of reproductive phenology or altering pollinator behavior (Aldridge and Campbell, 2007). In this case, prezygotic reproductive isolation would be altered. A third way of influencing hybridization is if a change in environmental conditions in an area of overlap alters the relative ability of hybrids to survive and/or reproduce in comparison with the parental species (Fig. 1C). Reduced viability and reduced reproduction (partial or complete sterility) are two mechanisms of postzygotic reproductive isolation (Stebbins, 1947). Either one could change with the environment, as illustrated by the genotype by environment interactions seen in both *Helianthus* and *Iris* hybrids (Arnold and Martin, 2010). A change in viability would influence whether mature hybrids are seen, and a change in reproduction would influence whether advanced generation hybrids are formed. Combinations of these mechanisms, as well as other effects on hybridization, may also occur.

One way to test for potential effects of climate change on hybridization is to manipulate environmental conditions, such as temperature or precipitation. Manipulations of environmental conditions have greatly informed our understanding of ecosystem-level responses including biomass, net primary

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⁴Author for correspondence (e-mail: drcampbe@uci.edu)

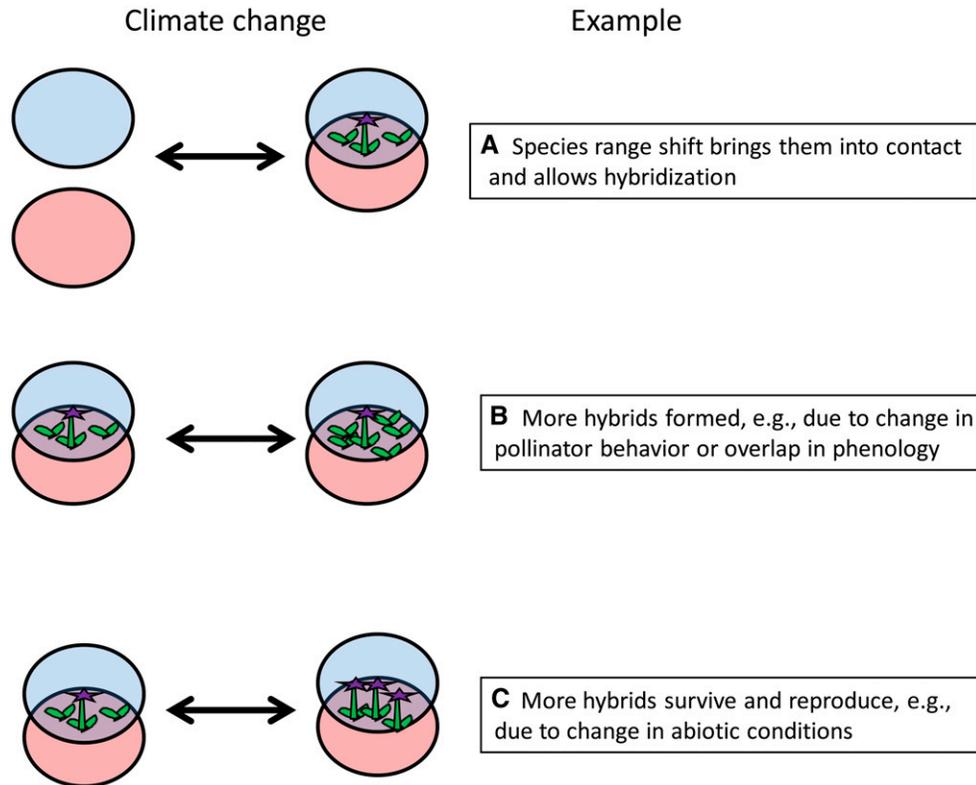


Fig. 1. Three theoretical ways in which climate change could alter a hybrid zone, singly or in combination. The blue and red ovals indicate the range of two species. Hybrid zygotes are illustrated as small seedlings, hybrids with high fitness as flowering plants. Either increases or decreases in hybrid frequency are possible with climate change, as indicated by double-headed arrows, but each example given is for an increase in hybrids. (A) Climate change increases the degree of spatial overlap, in this case from zero, by shifting the range of one species higher in latitude or altitude. (B) Prezygotic isolation is reduced in a zone of overlap, for example, by a change in pollinator behavior, such that more hybrid zygotes are formed. (C) More hybrids survive to reproduce or have higher reproductive success, for example, due to a change in abiotic conditions, such that postzygotic isolation is reduced.

production, and ecosystem photosynthesis (Wu et al., 2011). This approach of manipulating precipitation in the field has, however, not previously been applied to plant hybrid zones, although some recent studies have examined responses of hybrids to water manipulation under greenhouse conditions (Brock and Galen, 2005; Sherrard et al., 2009; Campbell et al., 2010; Ma et al., 2010). Spatial movement of hybrid zones and changes in introgression over historical time have been documented in several cases (Buggs, 2007), indicating the potential for such responses to future climate change. Here we examined the effects of manipulating precipitation in a common field garden on growth, leaf physiology, survival, and flowering of two parental species of the herbs *Ipomopsis* (Polemoniaceae) and their hybrids.

The sister species *Ipomopsis aggregata* (Pursh) V.E. Grant and *I. tenuituba* (Rydb.) V.E. Grant (Porter et al., 2010) form natural hybrid zones in many, but not all, areas where ranges overlap in the mountains of the western United States (Grant and Wilken, 1988; Aldridge, 2005). *Ipomopsis tenuituba* subsp. *tenuituba* is often found in drier habitat and has higher photosynthetic water-use efficiency than its congener *I. aggregata* subsp. *aggregata* (Wu and Campbell, 2006; Campbell and Wu, 2013). At the most intensively studied hybrid zone, hybrids experience even drier conditions and show even higher water-use efficiency (Campbell et al., 2005). In a dry-down experiment with potted plants, first and second generation hybrids with

I. tenuituba as the maternal parent contributing cytoplasmic genes had peak photosynthetic rate at a lower optimal soil water content than did the reciprocal hybrids (Campbell et al., 2010). At the best-studied hybrid zone, most natural hybrids have *I. tenuituba* cytoplasmic genes (Wu and Campbell, 2005). Together these studies suggest the hypothesis that imposition of drought conditions in the relatively more mesic environment where *I. aggregata* is typically found could favor a shift toward success of hybrids in comparison to *I. aggregata*. This prediction assumes that water-use efficiency correlates with fitness components in relatively dry habitats. The evidence for selection on intrinsic water-use efficiency, the ratio of carbon assimilated (A) to stomatal conductance (g), is still equivocal. Individuals with higher intrinsic water-use efficiency (hereafter WUE) under dry conditions achieved higher reproductive output in studies of *Cakile* (Dudley, 1996) and *Polygonum* (Heschel et al., 2004), but not in *Lobelia* (Caruso et al., 2006). Another functional trait that often correlates with moisture regime is specific leaf area (SLA = area / dry mass). SLA is generally higher for species in areas with more rainfall (Westoby and Wright, 2006), but its effect on plant fitness has rarely been measured (Agrawal et al., 2008), and it has not previously been studied in *Ipomopsis* hybrid zones.

We performed both additions and reductions of precipitation and examined responses of the two species and their natural hybrids during both the vegetative and flowering stages. Relative

fitness can depend not only on events during the vegetative stage but also on the expression of flowering traits. In *Ipomopsis* for example, plants with higher nectar production and wider corollas achieve higher pollination by hummingbirds (Mitchell, 1993; Campbell et al., 1996; Campbell et al., 1997). Any change in these floral traits with water availability might therefore influence hybrid fitness and hence postzygotic reproductive isolation. Nevertheless, responses of floral traits to water availability are not as well studied as vegetative traits. Greenhouse studies have often found reductions in nectar volume and flower size with imposition of dry conditions (Carroll et al., 2001; Halpern et al., 2010), but field tests of how precipitation affects such traits are rare (Wyatt et al., 1992; Galen, 2000).

Our study examined how precipitation influenced survival of the parental species and the relative fitness of hybrids under new environmental regimes, testing aspects of the mechanisms shown in Fig. 1A and Fig. 1C, respectively. We addressed the following questions:

(1) Does an increase or decrease in precipitation away from ambient levels differentially affect the leaf physiology (WUE and SLA) or growth of the two plant species and their hybrids? A change in leaf physiology would suggest a biological basis for how hybridization could respond to precipitation. Such a physiological change could potentially underlie either the ability of one of the parental species to exist in the new environment (Fig. 1A) or the ability of hybrids in particular to survive and reproduce (Fig. 1C).

(2) Does a change in precipitation affect reproductive traits and flower number as well as the vegetative responses more commonly measured in studies of global change? Reproduction is part of fitness, and if a species has higher or lower fitness components under an altered precipitation regime, this result would suggest the potential for a range shift with environmental change (Fig. 1A). For example, if *I. tenuituba* had low reproduction under the ambient conditions for *I. aggregata*, but both species had high reproduction under dry conditions, that scenario could increase the opportunity for hybridization in the case of drought.

(3) Do hybrids respond differently to precipitation from the parental species in term of survival and flower number? If the relative fitness of hybrids is greater under an altered precipitation regime compared to ambient, that would support a change in postzygotic isolation (Fig. 1C). We hypothesize that a reduction in precipitation would favor the success of hybrids over *I. aggregata*. Whereas our estimate of fitness did not include all components (e.g., from seed to vegetative rosette), observed impacts on survival and flower number provide a first step in understanding potential responses of hybridization to changes in precipitation.

MATERIALS AND METHODS

Establishment of common garden—We set up a common garden in a sub-alpine site at the Rocky Mountain Biological Laboratory (RMBL), Gunnison County, Colorado (CO), USA in summer 2009, a site where normally only *I. aggregata*, but not *I. tenuituba* or hybrids, is found. This garden contained plants of the two *Ipomopsis* species and natural hybrids, subject to three experimental conditions: ambient precipitation, additional precipitation, and reduced precipitation. The plants were transplanted as vegetative rosettes removed from two contact sites in Gunnison County, CO where natural hybrid zones occur. Plants were removed from populations of *I. aggregata*, *I. tenuituba*, and natural hybrids identified and described elsewhere for the contact sites of Poverty Gulch (Campbell et al., 1997) and Deadman's Gulch (Campbell and Wu, 2013).

Plants of these species are monocarpic, blooming once before dying, and take a median of 5 yr to bloom at Poverty Gulch (Campbell and Waser, 2007). When plants were collected, they were in their second or later summer of growth, as first year plants are distinguishable by cotyledons at this time in the season. From each of the two contact sites, we used 54 plants, 18 of each of the three types.

Because plants were started as rosettes rather than seeds, we took precautions to minimize the chance that responses would reflect differences in early growth conditions prior to the start of the experiment. First, we standardized the size of rosettes at the start of the study. Length of the longest leaf averaged 50 mm (SE = 1 mm), and number of leaves averaged 30 (SE = 1). Overall size, obtained by multiplying these two measurements (Campbell and Waser, 2001) did not differ significantly among the three plant types or the site of origin at the start of the study (both $P > 0.05$ in two-way ANOVA). Second, we used plants from two different contact sites that occupy different habitats. These two contact sites are sufficiently different that *I. aggregata* is the lower elevation species at the well-studied Poverty Gulch but the higher elevation species at Deadman's Gulch (Aldridge, 2005). Thus, any differential responses of the three types of plants should be generalizable across *Ipomopsis* contact sites.

Plants were potted from the source field sites between 25 June and 2 July, watered daily, and then transplanted into the garden on 3 July. The common garden consisted of nine plots, in a 3 × 3 arrangement, each 1 m² and separated from adjacent plots by a margin of 1 m. Plots were cleared of aboveground vegetation. Into each plot we transplanted 12 plants, two of each of the three types from each source site, in a 3 × 4 grid with spacing of 25 cm and positions assigned at random. All plots were watered daily until 10 July to facilitate establishment.

Precipitation manipulations were initiated on 11 July 2009 and continued through the summers of 2009 (11 July to 30 August), 2010 (23 June to 30 August), and 2011 (28 June to 18 August). One of the three plots in each row was assigned at random to the wet treatment, one to ambient, and one to the dry treatment. The entire design corresponded to a replicated split-plot design, with precipitation manipulated at the whole-plot level, and plant type manipulated at the within-plot level. This design is efficient for detecting plant type by treatment interactions, which were our main interest, but has less power for detecting main effects of the precipitation treatment. Plots in the wet treatment initially received 50% more water (in the form of tap water) than the historical average during July from 1989 to 2006 based on the EPA weather station at the Research Meadow at RMBL (http://rmbll.info/rockymountainbiolab/rdc/rdc_epa_sensing.html), but from 23 July 2009 on, they received double the historical average. This doubling of average water amounted to 3.5 L extra water every 2 d to each 1 m² plot. The three dry plots were covered with rainout shelters designed to intercept 50% of incoming summer precipitation. The rainout shelters measured 1.6 m on a side and were centered over the dry plots to minimize edge effects. The shelter was constructed from 2 × 2 strips of wood with a sloping partial roof consisting of U-shaped strips of clear, corrugated greenhouse roofing that covered 50% of the top area. Water intercepted by these strips fed into a plastic gutter (as in Yahdjian and Sala, 2002) and then into a bucket for removal. Shelters were open at the sides, allowing free access by pollinators and herbivores. The three ambient plots generally received only natural rainfall, except for 2 d during a dry period in early July 2009 when plants were still establishing and wet and ambient plots were provided with an additional 2.5 L and dry plots with 1.25 L of water. To assess the efficacy of our precipitation manipulations, we compared actual amounts of water received to the precipitation recorded at the RMBL during the same periods of 2009, 2010, and 2011. To monitor any impact of the rainout shelter on light levels, we suspended Hobo light data loggers (Onset Computer, Bourne, Massachusetts, USA) 30 cm above the center in two replicates of each treatment. Over the daylight hours of 0700 to 1700 hours, the plots with rainout shelters averaged 10% less light (64766 vs. 71984 lux) than the other plots, but this difference was not statistically significant (ANOVA, $F_{2,3} = 0.65$, $P = 0.58$). In early September of the first year, we placed poultry wire over all plants to prevent overwinter damage by ground squirrels and gophers. During summer of 2010, we began covering the wet and ambient treatments with sham rainout shelters identical to the dry treatment shelters, except with no plastic roofing, as the shelters reduced browsing by deer. With those sham shelters in place, any differences in light level among plots should have been even smaller than in 2009.

Plant response measurements—We measured vegetative traits and leaf-level physiology during the first summer of manipulation, flower traits and flower number during the second and third summers, and survival and size throughout the study. By the end of the study, all but six of these monocarpic plants had bloomed and died or died without blooming, so we were able to follow

the vast majority through their life cycle. To avoid genetic contamination due to using plants from distant localities, we emasculated flowers in the bud phase on all plants. Three times per week during the blooming season, we used fine forceps to remove anthers from elongated buds.

The size of the vegetative plant was determined by multiplying the length of the longest leaf by the number of leaves (after adding in the small number of leaves that had been removed already that season for analysis). For plants with multiple rosettes, size was determined separately for each rosette, and those values were added to obtain a measure of overall size (Campbell and Waser, 2001). We calculated relative growth rate during the first season by dividing the change in size between 7 July and 14 August by the initial size. Specific leaf area was measured during the first season of growth in the common garden. Fresh leaves were scanned and the areas determined with ImageJ (freeware from National Institutes of Health, Bethesda, Maryland, USA) before drying to a constant mass in a 60°C oven. We averaged the SLA for two leaves collected respectively 11–12 d and 24–25 d after precipitation treatments began.

Leaf gas exchange was measured using a Li-Cor 6400 Portable Photosynthesis system (Li-Cor, Lincoln, Nebraska, USA). All measurements were made between 0800 to 1200 hours with saturating light conditions (PAR = 1800), a leaf temperature of 27°C, and sample CO₂ concentration of 375 ppm (following Wu and Campbell, 2007). Intrinsic water-use efficiency was calculated as A/g , where A is photosynthetic rate and g is stomatal conductance, with values corrected for area inside of the leaf chamber. Gas exchange was measured three times on different leaves of each plant throughout the first season (13–15 July, 29–31 July, and 10–12 August), and average values were used in analysis. At each time that gas exchange was measured, we also measured soil moisture as volumetric water content (VWC) using a 12-cm probe (Campbell Scientific, Edmonton, Alberta, Canada) inserted in the ground near each plant. Average VWC values for plants were analyzed with ANOVA with the factors of treatment and plot nested within treatment.

In the two succeeding years, we recorded survival and flowering. Survival to flowering was censused on 20 June 2010 and 22 June 2011. For each plant that bloomed, we assessed flower number in addition to other floral traits. Corolla length and width at the opening were measured on an average of 4.3 flowers per plant, and plant means were used in analysis. Plant values for 48-h nectar production and percentage sugar concentration (both measured as described by Campbell et al. [1991]) were based on an average of 3.3 flowers per plant. Number of flowers produced by the plant over its lifetime were obtained by counting all reproductive structures (buds, flowers, fruits, aborted fruits) on each plant at the end of its one blooming season. The calyces of flowers remain on the plant after seeds are shed, allowing fruits to be counted at this time. Because all plants were emasculated to prevent genetic contamination, pollen availability for fertilizing seeds was low in the vicinity, requiring us to use flower number rather than seed production as an index of reproductive success. Flower number correlates highly with seed production in *Ipomopsis aggregata* (Campbell, 1989). We also determined aboveground and belowground biomass for the flowering plants. Plants were carefully extracted from the soil after flower counts were complete, and soil was washed from the fine roots. Above- and below-ground parts were weighed separately after drying to a constant mass in a 60°C oven. Our root extractions revealed minimal overlap in root zones of plants, which along with lack of aboveground overlap suggests that competition was minimal.

Statistical analysis of plant responses used a split plot model with treatment as a fixed factor, plot nested within treatment, type of plant as the within-plot factor, and the treatment × type interaction. For vegetative and physiological traits, residuals were approximately normally distributed. A differential effect of precipitation on leaf physiological traits (question 1) was tested by the treatment × type interaction. The main effect of treatment, and the interaction of treatment with plant type, on floral traits tested question 2. To test question 3, we compared fitness of hybrids to that of the parental species. As an index of fitness, we used total number of flowers produced, which in turn equals survival to reproduction × flowers per reproductive plant. For the fitness index, we used a zero-inflated Poisson model in Proc Genmod of the program SAS version 9.2 (SAS Institute, Cary, North Carolina, USA) and likelihood ratio statistics for type 3 analysis. The zero inflation portion of the model analyzed whether the plant survived to produce any flowers or not, giving the same result as a logistic model with binomially distributed errors, whereas the Poisson portion of the model analyzed flower count given that the plant reproduced. A differential effect of precipitation on survival or reproductive success would be indicated by a treatment × type interaction. To test specifically whether a reduction in precipitation favors hybrids over *I. aggregata*, thereby reducing postzygotic isolation (question 3), we used an a priori contrast within a zero-inflated Poisson model that compared the relative success of *I. aggregata* and hybrids under reduced precipitation to their relative success under ambient conditions.

RESULTS

Precipitation treatments and soil moisture—During the period of manipulation that was common to all 3 yr (11 July to 16 August), ambient plots received average precipitation slightly less than the historical average over the past 23 yr (71 vs. 91 mm; Fig. 2). The wet plots were above the historical average, and the dry plots below the historical average, for all 3 yr (Fig. 2). The wet plots received 3.9, 3.1, and 5.4 times as much precipitation as the dry plots in 2009, 2010, and 2011, respectively, averaging close to our treatment goal of a 4-fold range in precipitation. In 2009, the treatments led to 7.4%, 10.8%, and 14.2% average volumetric water content in the soil for dry, ambient, and wet plots, with significant differences among the treatments ($F_{2,6} = 24.15$, $P = 0.0013$). Thus, the treatments were effective at creating a gradient in soil moisture.

Effects on vegetative traits—These precipitation treatments impacted the relative growth rate of the two *Ipomopsis* species differentially. A split-plot ANOVA showed a treatment × plant type interaction ($F_{4,79} = 3.33$, $P = 0.0142$; Table 1) and a trend toward overall increased growth with increased precipitation ($F_{2,6} = 3.98$, $P = 0.0793$). Growth of *I. aggregata* and hybrid rosettes was relatively insensitive to the precipitation treatment, in comparison to growth of *I. tenuituba*, which was high only in the wet treatment, even though *I. tenuituba* is the species typically found in drier conditions (Fig. 3A). Separate analysis by treatment revealed that *I. aggregata* had a higher relative growth rate than *I. tenuituba* during the first season in both the dry treatment (contrast comparing species, $F_{1,23} = 6.85$, $P = 0.0154$) and the ambient treatment ($F_{1,31} = 3.98$, $P = 0.0550$) but not in

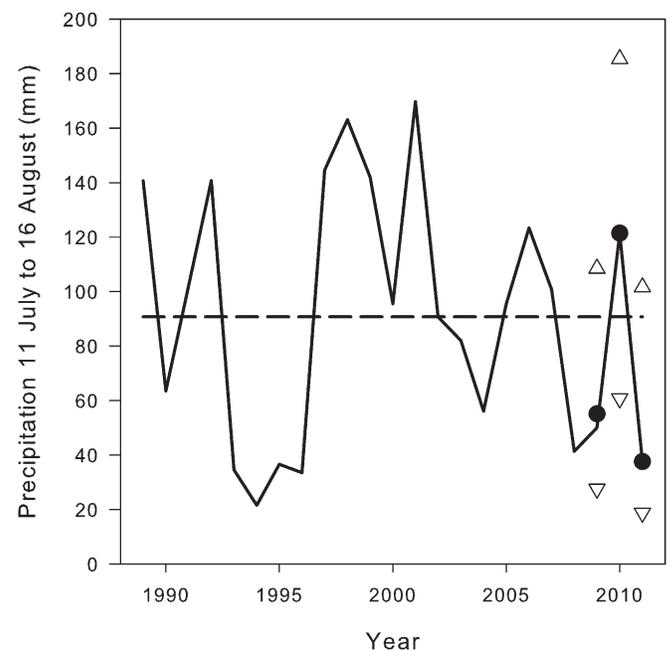


Fig. 2. Precipitation over 23 yr during the period of manipulation maintained in all 3 yr of the experiment (11 July to 16 August). Records were obtained from the EPA data set for the Research Meadow at the Rocky Mountain Biological Laboratory. The dashed line indicates the mean precipitation across years. Solid circles: precipitation in ambient plots during 2009–2011. Downward triangles: precipitation in dry plots during 2009–2011. Upward triangles: precipitation in wet plots during 2009–2011.

TABLE 1. Results of split-plot analysis of variance for plant responses analyzed with Proc GLM of SAS ver. 9.2 using type III SS.

Response variable	Source of variation							
	Treatment		Plot (Treatment)		Type of plant		Treatment × Type	
	F	P	F	P	F	P	F	P
RGR	$F_{2,6} = 3.98$	0.0793	$F_{6,79} = 1.48$	0.1967	$F_{2,79} = 1.83$	0.1669	$F_{4,79} = 3.33$	0.0142
SLA	$F_{2,6} = 0.05$	0.9510	$F_{6,85} = 1.71$	0.1293	$F_{2,85} = 2.79$	0.0668	$F_{4,85} = 0.59$	0.6716
A/g	$F_{2,6} = 2.27$	0.1843	$F_{6,79} = 7.77$	0.0001	$F_{2,79} = 0.52$	0.5941	$F_{4,79} = 4.01$	0.0052
Flowers per repr.	$F_{2,6} = 0.68$	0.5396	$F_{6,46} = 1.71$	0.1406	$F_{2,46} = 2.71$	0.0770	$F_{4,46} = 4.53$	0.0036
Corolla length	$F_{2,6} = 5.29$	0.0474	$F_{6,44} = 0.53$	0.7803	$F_{2,44} = 0.92$	0.4067	$F_{4,44} = 0.30$	0.8767
Corolla width	$F_{2,6} = 0.77$	0.5038	$F_{6,44} = 1.33$	0.2629	$F_{2,44} = 21.42$	<0.0001	$F_{4,44} = 0.22$	0.9269
48-h Npr	$F_{2,6} = 1.35$	0.3273	$F_{6,43} = 0.47$	0.8290	$F_{2,43} = 44.87$	<0.0001	$F_{4,43} = 0.05$	0.9944

Notes: RGR = relative growth rate, SLA = specific leaf area, A/g = intrinsic water-use efficiency, Flowers per repr. = flowers per reproductive plant, Npr = nectar production rate. Significant effects are in boldface.

the wet treatment ($P > 0.10$). For SLA, we saw no differential effects of the precipitation treatment on the three types of plants (interaction NS; Table 1) and little sign of plasticity in general (treatment effect NS). Plants of *I. aggregata* had consistently higher SLA than plants of *I. tenuituba*, the species coming from drier habitats (Fig. 3B; contrast comparing species; $F_{1,85} = 4.89$, $P = 0.0297$). Averaging across the treatments, hybrids were intermediate to, and did not differ significantly from the average of, the two parental species for either relative growth rate or SLA (contrast $P = 0.56$ and 0.39 , respectively).

In contrast, the precipitation treatments markedly changed the WUE of hybrids relative to the parental species. There was a significant interaction between treatment and plant type, when all three types were included in the model ($F_{4,79} = 4.01$, $P = 0.0052$). Although all plant types were able to increase WUE to some extent in the dry treatment, the hybrids ramped up WUE to a greater extent (Fig. 3C). This higher WUE resulted primarily from a change in stomatal conductance; hybrids in the dry treatment had the lowest conductance of all type/treatment combinations, whereas photosynthetic rate for those plants was not unusually high. Analyzing the data separately by treatment, in the dry conditions the hybrids had higher WUE than the average of the parental types (Fig. 3C; contrast $F_{1,22} = 8.09$, $P = 0.0095$). In the other two treatments, no significant differences were detected among the three plant types ($P > 0.05$). Unlike relative growth rate, which mainly showed differences between the responses of the two parental species to precipitation, WUE showed a change in hybrid performance with precipitation.

Effects on fitness components and floral traits—Survival to flowering varied among types of plants (main effect of type in zero-inflation portion of model, χ^2 with 2 df = 6.88, $P = 0.0321$). At this home site for *I. aggregata*, plants of *I. aggregata* had the highest success at surviving to flowering (69%), whereas *I. tenuituba* had the lowest (44%; Fig. 4A). Hybrids responded dramatically differently from the parental species to precipitation in terms of number of flowers produced by those plants that survived to blooming (Fig. 4B). Whereas *I. aggregata* generally increased flower production with increased moisture, the hybrids flowered especially profusely under the reduced precipitation treatment, leading to a plant type × precipitation interaction (χ^2 with 4 df = 1001.50, $p < 0.0001$ in the zero-inflated Poisson model, or $P = 0.0036$ for a standard split-plot ANOVA model; Table 1). Overall, the relative fitness index for *I. aggregata* compared to hybrids switched from favoring *I. aggregata* under ambient conditions to favoring hybrids under reduced precipitation (Fig. 5; contrast $\chi^2 = 486.63$, $p < 0.0001$). This effect was largely due to the switch in

relative number of flowers per survivor made by the two types of plants when conditions were changed from ambient to dry (contrast $F_{1,52} = 9.82$, $P = 0.0028$). A similar switch in the relative number of flowers by the two pure species was not seen between ambient and dry conditions (contrast $P = 0.36$) nor between ambient and wet conditions (contrast $P = 0.19$). Despite the treatment × type interaction for flower number, we detected no effects on aboveground biomass of reproductive individuals (split-plot ANOVA, all $P > 0.05$) or on belowground biomass (all $P > 0.05$).

The only flower trait to respond detectably to the precipitation treatment was corolla length ($P = 0.0474$; Table 1). An increase in water generated longer flowers for all plant types (Fig. 4C). Nectar showed on average higher 48-h volume and lower concentration in the wet treatment (3.8 μ L and 22.2% sucrose content) compared to the dry treatment (3.3 μ L and 24.9%), but in neither case was the effect of treatment significant (Table 1). Plants of the three types differed significantly in corolla width and nectar production (both $P < 0.0001$), with the wider flower and greater nectar production characteristic of *I. aggregata* (Campbell and Aldridge, 2006) retained across all treatments. None of the flower traits showed a plant type × precipitation interaction (Table 1).

Since our results demonstrated differential effects of precipitation on relative growth rate during the first season, intrinsic water-use efficiency, and number of flowers by reproductive plants, we explored the relationships between these variables using path analysis. We used structural equation modeling with Proc Calis in SAS to implement the paths shown in Fig. 6. For all flowering plants analyzed together, all of the paths except for the one from soil moisture to relative growth rate were statistically significant ($P < 0.05$). Both WUE and relative growth rate led to increases in flower number, with an overall negative correlation between these two predictors (Fig. 6). Overall, the path from WUE to flower production had the highest standardized estimate ($0.47 \pm$ SE of 0.12). The relative importance of the paths through WUE and relative growth rate, however, differed by plant type. Hybrids had the strongest paths through WUE, with decreased soil moisture leading to an increase in WUE, which in turn was positively related to flower production (Fig. 6). In contrast, plants of *I. tenuituba* showed a higher negative correlation between relative growth rate and WUE, with higher growth rate positively related to flower production.

DISCUSSION

Effects of precipitation on hybrid fitness and reproductive isolation—By following responses to experimental alterations

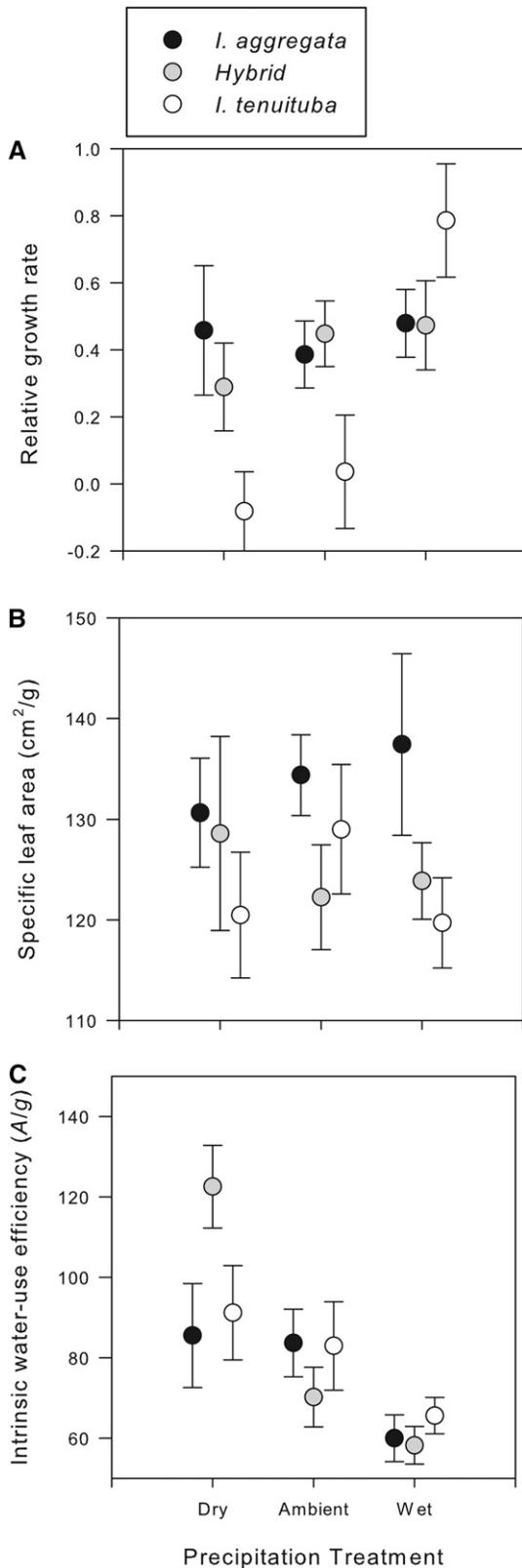


Fig. 3. Responses of the two species of *Ipomopsis* and natural hybrids to precipitation during the first season of vegetative growth. Means \pm SE are shown. (A) Relative growth rate over 38 d. (B) Specific leaf area. (C) Intrinsic water-use efficiency (A/g).

of precipitation level over 3 yr, we were able to assess the relative performance of these two species of *Ipomopsis* and natural hybrids at both the vegetative and reproductive stages. Our first question asked about changes in growth and physiology with manipulation of precipitation. The growth of vegetative rosettes responded more dramatically to an increase in water in *I. tenuituba* than in the other two types of plants. Although most ecosystem level studies show strong effects of changing precipitation on aboveground biomass and aboveground net primary productivity (Wu et al., 2011), the present study illustrates that growth effects are highly species-specific. Furthermore, whereas all types of plants increased intrinsic WUE under artificially imposed drought, as is commonly seen (Heschel et al., 2004; Sultan et al., 1998), the *Ipomopsis* hybrids did so to a greater extent. Previous experimental studies of drought imposition on subalpine or alpine plants, done in the greenhouses, have shown conflicting results, with greater plasticity of WUE in response to soil moisture for the homoploid hybrid *Pinus densata* compared to the parental species (Ma et al., 2010), but not in the case of *Taraxacum* hybrids (Brock and Galen, 2005).

Second, we asked whether there were responses to precipitation during the reproductive as well as the vegetative stage. The two parental species did not show differential responses of floral traits to the precipitation regime. The hybrids, however, produced more flowers compared to the two parental species under drought conditions. This excess flower production was highly correlated with their increase in WUE, as indicated by the path analysis. Despite production of more flowers, the only individual flower trait that responded detectably was corolla length. There were no detectable effects on nectar volume or corolla width, the two floral traits we measured with known influences on pollination success, suggesting that altered precipitation in this case would not markedly influence reproductive isolation via a mechanism of altering these particular floral traits. The lack of an effect of 4-fold variation in precipitation on nectar volume in the field is consistent with two previous experimental studies of the species *I. aggregata* (Campbell and Halama, 1993; Burkle and Irwin, 2009), although in severe droughts when soil moisture below 5% has been recorded, this species has stopped nectar production entirely (N. Waser, University of Arizona, personal communication).

For the two parental species of *Ipomopsis*, altering precipitation did not influence their relative fitness (based on survival and flowers per survivor). Thus, there was no evidence that this degree of change in precipitation would alter the ability of the two parental species to occupy the same habitat in sympatry, as shown in Fig. 1A. Plants of *I. tenuituba* were able to survive and reproduce to a similar extent in all three precipitation regimes, a surprising result in light of the absence of this species in the habitat where the experiment took place, and indeed in the entire East River valley.

Finally, we asked how the relative fitness of hybrids changed with precipitation. The relative success of hybrids was greater under experimentally imposed drought, supporting a change in postzygotic isolation with that environmental change, as illustrated in Fig. 1C. The hybrids responded differently from their parents largely in terms of flower number rather than survival to reproduction. In particular, a reduction in precipitation favored the success of hybrids over *I. aggregata* as we had predicted from earlier studies of leaf gas exchange in natural hybrid zones (Campbell et al., 2005). An increase (as opposed to a decrease) in precipitation had no impact on the relative fitness index for

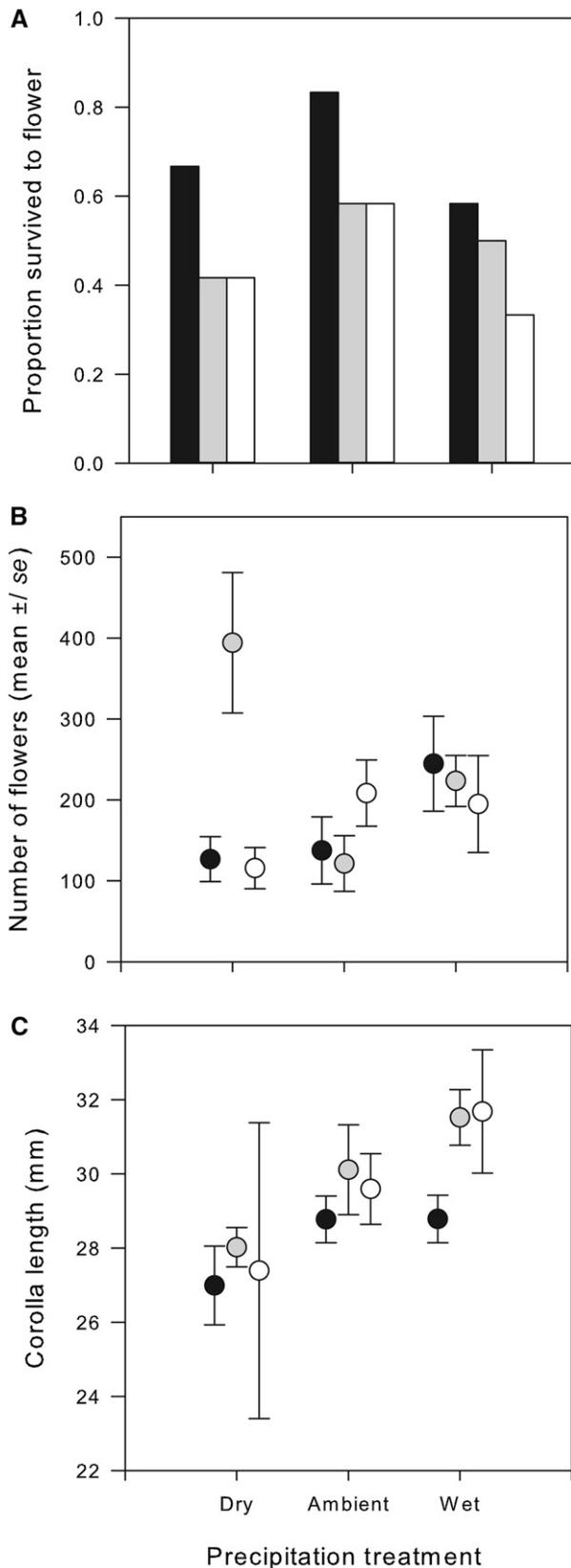


Fig. 4. Reproductive responses to the three precipitation treatments. Error bars indicate one SE. (A) Proportion of plants surviving to flower. (B) Flowers per reproductive plant. (C) Corolla length of flowers.

I. aggregata vs. hybrids. Thus, the responses to the two kinds of changes in precipitation were asymmetrical.

Potential impacts of droughts on *Ipomopsis* hybrid zones—

Taken at face value, these results suggest that if climate change were to generate more droughts in the Colorado Rockies, then hybrids between these two *Ipomopsis* species may become more common due to an increase in fitness (and thus a decrease in postzygotic reproductive isolation). There is considerable uncertainty surrounding predictions for mean summer precipitation in the southwestern United States (Notaro et al., 2012). Effects on variation in precipitation are, however, likely to be more important than effects on the mean for *Ipomopsis*, because the greater response to a reduction vs. an increase in precipitation suggests that success of *I. aggregata* relative to hybrids over a number of years would decline with increasing environmental variation (Drake, 2005). When precipitation fell below about 42 mm during a 36-d period of study (mean of dry treatment in Fig. 2), hybrids produced more flowers, but when precipitation was increased their flower production did not differ from that under ambient conditions. Thus, it is the frequency and intensity of droughts that will affect the relative performance of these hybrids. The projected warming in this area is likely to intensify droughts by increasing evapotranspiration, and droughts are projected to increase in severity and duration (Notaro et al., 2012).

Even if intense droughts become more common, however, making a prediction about how *Ipomopsis* hybrid zones would respond would necessitate three important untested assumptions. First, we would need to assume that counterbalancing effects on fitness do not take place during the portions of the lifecycle that we did not follow, from seed to vegetative rosette and from flower production to seed production. A previous study examined survival of these two species and their hybrids started from seed in three common gardens and found survival differences among the parental types and hybrids starting only at age 3 (Campbell and Waser, 2001). Furthermore, these types of plants did not differ significantly in seed mass, and potential maternal effects mediated by seed mass did not help to explain variation in survival or growth among the types of plants. That study suggests that it is unlikely we missed important genotype by environment interactions by omitting that early stage. Although we cannot rule out maternal effects in our study, the absence of explanatory maternal effects in the earlier study, along with our use of similarly sized plants from two different contact sites, also suggests it's unlikely that observed differences between plant types in response to precipitation reflected maternal environmental effects. However, there still could be overall responses to drought imposition in the early life history, because percentage seedling emergence of *I. aggregata* in a given year correlates with precipitation during May and June (Waser et al., 2010).

The potential effects of a prolonged drought on events during the other stage we missed (flower to seed production) are harder to predict, as these would depend not only on the ability of hybrids to mature seeds but also on responses of the animal pollinators to dry conditions. In terms of ability to mature seeds, hand-pollinations between F1 hybrids are equally as successful as the average of conspecific crosses at producing seeds (Campbell et al., 2008). The most common pollinators are hummingbirds and hawkmoths, although some visits are also made by solitary bees and butterflies as well as the occasional other insect (Price et al., 2005). We saw no strong effects of precipitation on two flower traits, nectar production and corolla width,

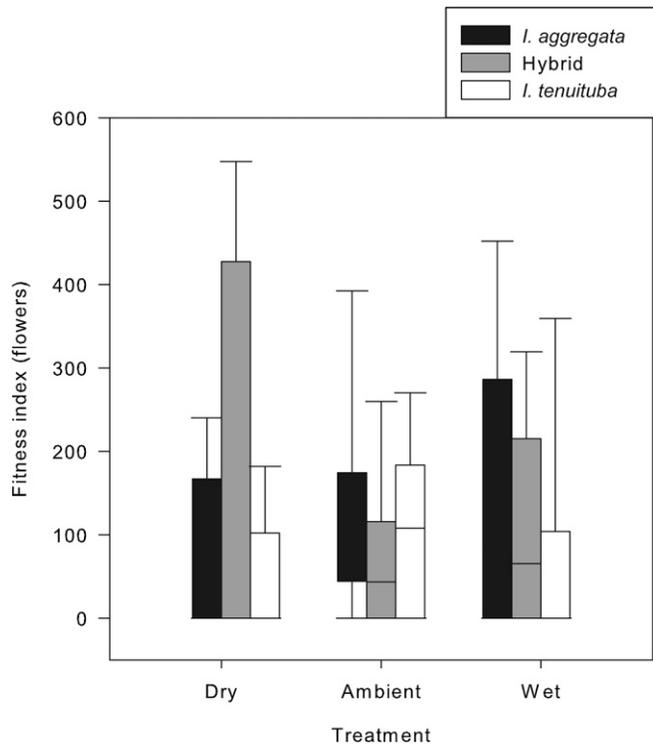


Fig. 5. Box plots showing the overall fitness index for the two species of *Ipomopsis* and natural hybrids under three precipitation regimes. The fitness index (flowers) equals survival to flowering (shown in Fig. 4A) multiplied by number of flowers per reproductive plant (Fig. 4B). Lines show medians, and points indicate the 5th and 95th percentiles.

that the hummingbird and hawkmoth pollinators are known to respond to in *Ipomopsis*. However, we simply do not know how populations of these animal pollinators respond to drought.

A second assumption, even if we restrict predictions to impacts of changes in summer precipitation only, is that effects of summer precipitation do not interact with those of other climatic variables, such as winter precipitation and temperature. A reduction in winter precipitation accelerates the phenology of many flowering plant species in the mountainous area of our study by altering the timing of snowpack melt (Inouye, 2008; Forrest et al., 2010). It is conceivable that plants that flower earlier would be more prone to impact by summer droughts, especially since June is a drier month than July in this area. Furthermore, responses to summer drought could depend also on changes in temperature. Hybrids of these two species of *Ipomopsis* have an optimal temperature for photosynthesis that is higher than that of *I. aggregata* but lower than that of *I. tenuituba* (Wu and Campbell, 2006). If temperature increased along with drought stress, we might then expect hybrids to continue to perform physiologically better than *I. aggregata*, but we do not know how such a difference in the photosynthesis to temperature relationship affects either survival or reproductive success. In ecosystem-level studies, the combined effects of manipulating temperature and precipitation have tended to be less than predicted by adding together the effects of single manipulations (Wu et al., 2011). Similar combinations of manipulations should also be tried in future attempts to predict evolutionary responses of plants to climate change.

Finally, hybrids would only become more common with drought if the observed effects on postzygotic isolation were not cancelled out by opposing effects on prezygotic isolation, such as shown in Fig. 1B. Some of our previous studies have suggested the hypothesis that warmer temperatures might actually increase prezygotic isolation. At a warmer contact site between the two species in Montrose County, Colorado, hybrids are rare in part because the same pollinators found in Gunnison County are more highly species-specific in foraging than they are at cooler sites, with the hawkmoth *Hyles lineata* visiting *I. tenuituba* nearly exclusively (Aldridge and Campbell, 2007).

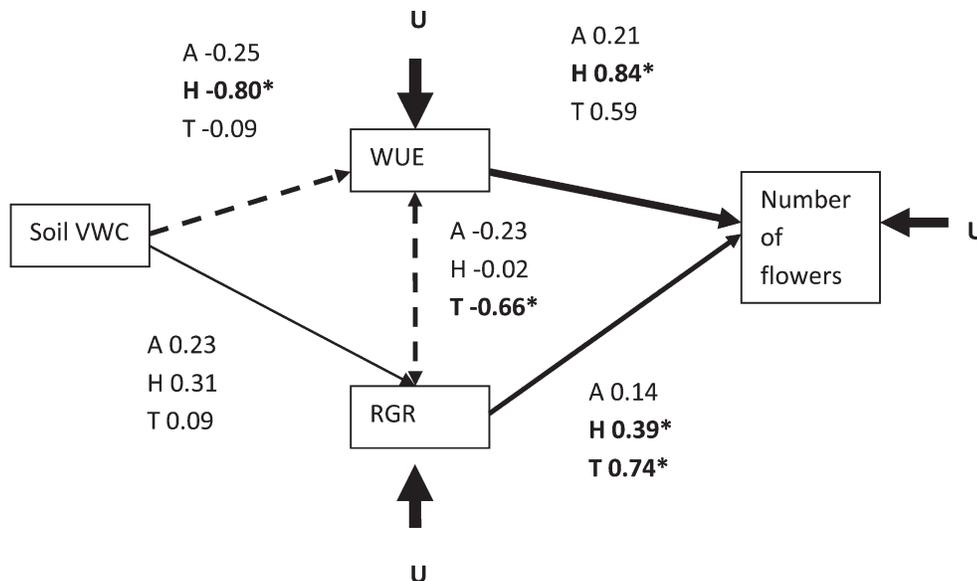


Fig. 6. Path analysis showing relationships between soil moisture (% VWC), relative growth rate over 38 d (RGR), intrinsic water-use efficiency (WUE), and the number of flowers for plants that survived to flowering. The width of the arrows is proportional to the standardized estimate of the path for all plants combined. Dashed lines indicate negative path coefficient estimates. U indicates unexplained source of variation. Numbers give standardized estimates when each type of plant (A = *I. aggregata*, H = hybrid, T = *I. tenuituba*) was analyzed separately. * $P < 0.05$.

We hypothesized that this high species-specificity at the warmer site may result from a shift to crepuscular (dusk and dawn) rather than daytime foraging by the hawkmoths, in turn associated with the warmer conditions (Aldridge and Campbell, 2007). After dark, the pale flowers of *I. tenuituba* may differ more in visibility to hawkmoths from the red-flowered *I. aggregata*. In addition, at night *I. tenuituba* produces a floral volatile, indole, that is often found in sphingid-pollinated flowers (Dobson, 2006), only after dusk (M. Bischoff, A. Juergens, D. R. Campbell, unpublished manuscript). The possibility that behavior of animal pollinators might change to increase prezygotic reproductive isolation, while direct effects of drought on plant performance decrease postzygotic reproductive isolation, highlights the need to consider interactions with other species in predicting responses of plant species to climate change.

Conclusions and lessons for studies of climate change—Although this study examined only a small portion of the ways in which an environmental change (in this case altered precipitation) could affect hybridization, the results offer some lessons for further studies of plant responses to global change. First, the results show the potential for changes in one kind of evolutionary response, the fitness of hybrids and therefore the degree of reproductive isolation between species. Although responses of plant hybridization to direct manipulation of precipitation have not previously been studied, this work adds to other kinds of evidence that changes in moisture regime can influence hybridization. For example, adaptive introgression of traits from *Helianthus debilis* for rapid growth and drought escape are associated with historical expansion of *H. annuus* into drier habitats (Whitney et al., 2010b). Our study also joins other recent studies (Hoffmann and Sgro, 2011) in pointing to the need to examine genetic variation and potential evolutionary changes in predicting impacts on species ranges, as has recently been done for *Arabidopsis thaliana* (Banta et al., 2012). Second, the asymmetrical response of hybrids to precipitation, with a far greater response to a reduction in precipitation than to an increase, suggests that a change in the variance of environmental conditions could cause an evolutionary response even in the absence of a change in mean. Third, this study illustrates the value of following plant responses to environmental variables (e.g., temperature, precipitation, and CO₂) through the flowering as well as the vegetative stages of plant growth. Even though the fitness difference between the hybrids and the home species of *Ipomopsis aggregata* appeared to be associated with differences in water-use efficiency, an effect on a component of fitness did not show up until the flowering stage. If we had only examined effects on vegetative growth or on biomass, and not counted flowers, we would not have detected the advantage of hybrids in dry conditions. The results underscore the need to measure traits allied to fitness such as survival and number of offspring, rather than relying on biomass, to understand evolutionary responses to global change.

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