Elevational and Temporal Variation in *Ipomopsis* floral and Vegetative Traits

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**Abstract**

Exploring the geographic and temporal shifts of hybrid zones provides practical evidence for conservation biologists investigating the dynamics of species invasions (Aldridge and Campbell 2006, Buggs 2007, Ellstrand 1992, Endler 1977, Rhymer and Simberloff, 1996). I measured *Ipomopsis* floral and vegetative traits in 12 populations along an elevational gradient near Gothic, Colorado. Plants in this population were measured in the early 1990s and in 2015, allowing me to track trait changes over time. Corolla length increased by an average of 4.4mm, and corolla width increased by 0.11mm between 1992 and 2015. Reciprocal transplant experiments found genetic and environmental bases for variation in corolla length and anther position. Genetic-based variation in the corolla length over 23 years could represent evolutionary change and a plastic response to environmental variation. Contrastingly, corolla width did not change systematically over time. Floral trait variation in this hybrid zone involves a complex assortment of selection pressures, mediated by multiple pollinators and varied environmental conditions, which may be the cause of differential change in floral traits throughout the cline.

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Elevational and temporal variation in *Ipomopsis* floral and vegetative traits

by

Gabriel Trujillo

April 12, 2016

The report of the investigation undertaken as a Senior Thesis, to carry two courses of credit in the Department of Biology.

Michael T. Orr
Krebs Provost and Dean of the Faculty

(Lynn Westley, Chairperson)

Glenn Adelson

Sean Menke
Abstract

Exploring the geographic and temporal shifts of hybrid zones provides practical evidence for conservation biologists investigating the dynamics of species invasions (Aldridge and Campbell 2006, Buggs 2007, Ellstrand 1992, Endler 1977, Rhymer and Simberloff, 1996). I measured *Ipomopsis* floral and vegetative traits in 12 populations along an elevational gradient in near Gothic, Colorado. Plants in this population were measured in the early 1990’s and in 2015, allowing me to track trait changes over time. Corolla length increased by an average of 4.4mm, and corolla width increased by 0.11mm between 1992 and 2015. Reciprocal transplant experiments found genetic and environmental bases for variation in corolla length and anther position. Genetic-based variation in the corolla length over 23 years could represent an evolutionary change and a plastic response to environmental variation. Contrasting, corolla width did not change systematically over time. Floral trait variation in this hybrid zone involves a complex assortment of selection pressures, mediated by multiple pollinators and varied environmental conditions, which may be the cause of differential change in floral traits throughout the cline.
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Dedication

I dedicate my thesis to my mother, Gloria Trujillo, who gave me great encouragement to complete my undergraduate education.
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Overview

The study of natural variation in floral traits is important because it helps us understand the roles of natural selection, gene flow, and genetic drift in shaping levels of adaptation and distribution of species (Jonas and Geber 1999). Evidence of local adaptation demonstrates the significance of past evolutionary processes and suggests a key role in plant responses to current ecological dynamics (Pratt and Mooney 2013). Intraspecific clinal variation in plant traits has been observed along elevational gradients in field conditions (Vitasse et al. 2014). Consistent abiotic gradients have been shown to drive genetically based clinal variation in morphology and phenology within a species (Pratt and Mooney 2013). However, quantitative variation can also be the result of plasticity and does not necessarily represent genetic variation.

Investigating genetically based clines, specifically, can also help uncover adaptive responses to climate change. Even though local adaptation of plant species has been widely studied, less is known about plasticity in plant traits and variation across species ranges (Leimu and Fischer 2008). A more variable environment should be associated with greater variation in plasticity (Levins 1968, Moran 1992). Key spatial patterns often involve a change in specific traits over an elevational gradient (Scheepens et al. 2010). A sharp change in a trait along an elevational gradient could indicate stronger natural selection on that trait.

My study system consisted of two species of *Ipomopsis* (*I. aggregata* and *I. tenuituba*) and fertile hybrids between them, which grow along an elevational gradient at Poverty Gulch, Gunnison County, CO. *Ipomopsis aggregata* grows in the valley at elevations of 2900 m and below, while *I. tenuituba* can be found on
steep slopes above 3100 m. Natural hybrid populations grow on dry slopes at intermediate elevations between the two parental ranges (Campbell, 2004). This environment transitions from flat flower meadows to steep rocky slopes with sparse vegetation.

Elevational differences within our study sites are associated with water availability. Lower elevations generally have greater soil moisture than higher elevations. Vegetative and physiological traits, such as specific leaf area (SLA) and water-use efficiency, are generally expected to experience different selection pressures in dry versus wet habitats (Dudley 1996). However, instantaneous measure of water use efficiency \( (A/E) \), photosynthetic rate \( (A_{\text{max}}) \) and transpiration rate \( (E) \) showed no significant correlation with corolla length or width in *Ipomopsis aggregata* at Poverty Gulch (Price et al. 2005). Another study at Rocky Mountain Biological Laboratory (RMBL), however, demonstrated that *I. aggregata* increased flower production with increased soil moisture, and that corolla length increased with greater water availability for all three plant types (*I. aggregata, I. tenuituba* and hybrids) (Campbell and Wendlandt 2013). Also, average floral morphology did not change in a plastic way with snowmelt date (Campbell and Powers 2015). These data may indicate that changes in elevational patterns over time could reflect both genetic differences and phenotypic plasticity. Genetic differences could be driven by natural selection or by temporal changes in the extent of hybridization. Differences in natural selection across the gradient could be produced by differences in water availability, but also by differences in the abundance of specialized pollinators associated with parental hybrid species; in this case, hummingbirds versus hawkmoths (Campbell et al. 1997). Overall, many contributing factors can influence floral and vegetative traits within this
system. However within this system, trait variation is observed to be associated with soil moisture and pollinator abundance. In order to understand how these factors influence floral morphology, long-term experiments must be conducted to record variation in trait response through space and time.

Spatio-temporal variation in selection has received relatively little attention, often due to a lack of long-term data (Siepielski et al. 2009). With a dataset dating back 20 years, my comparison of cline variation in floral traits has the potential to describe how these traits have evolved through time. Exploring the geographic shift in hybrid zones also provides practical evidence for conservation biologists investigating the dynamics of species invasions (Buggs 2007). Evaluating causes and consequences of hybrid zone change is critical if potential extinction is to be averted (Rhymer and Simberloff, 1996), as many threatened species owe their demise, in part, to hybridization with invading congeners (Allendorf and Lundquist 2003, Buggs 2007, Wolf et al. 2001). Hybrid zone movement is an expected response to changing environments through a change in spatial limitations and the responding expression of plastic and canalized phenotypes.

Plastic responses to precipitation have been shown to contribute to the processes of natural selection, speciation, and macroevolution (Scheiner, 1993; Crispo, 2008). For example, in *Atriplex hymenelytra, Encelia farinose, Encelia californica,* and *Salvia leucophylla* specific leaf area (SLA=leaf area/dry mass) demonstrates plasticity in response to altered precipitation (Knight and Ackerley, 2003), an adaptive response to variation in water availability. Plants growing under dry conditions are likely to have a lower SLA, as greater surface area also results in increased water loss through transpiration. However, increased surface
area also allows for maximized light collection and gas exchange, and therefore can be an advantage when water is not a limiting resource (Cornelissen et al. 2003). Examining the underlying mechanisms behind these plastic responses can illuminate and or predict which traits are selected for and, thus, which factors influence spatial and temporal shifts.

This study began by addressing the following question: How do present day elevational clines in corolla length, corolla width, and maximum anther position compare with those observed in 1991 and 1992 (Campbell et al. 1997) for populations of *Ipomopsis* located in Poverty Gulch, Gunnison County, Colorado? To supplement these data, SLA was measured to compare a cline in a vegetative trait with clines in floral traits in 2015. Based on known patterns of specific leaf area, I predicted that populations of *Ipomopsis* would exhibit higher SLA in sites with wetter soils, and lower SLA in sites with drier soils. I hypothesized that selection on vegetative traits is expected to differ from that of floral traits. This may be the case because environmental conditions are not extremely varied among populations along the elevational cline. Floral selection pressures, however, are determined, at least in part, by three pollinator species, only one of which is consistently abundant from year to year. By investigating temporal floral trait variation, I can see if there have been cline-wide systematic changes or if trait variation is random from year to year.

To explore the potential causes of temporal shifts in clines, I used reciprocal transplant experiments to answer the following question: To what extent are elevational differences in anther position, corolla length, and corolla width due to phenotypic plasticity versus genetic differences? Based on known patterns of floral traits, I predicted that populations of *Ipomopsis* would exhibit
relatively short and wide corollas in the species *I. aggregata* at lower elevations, while *I. tenuituba* should express long narrow corollas at higher elevations (Campbell 2004). These predictions are based on *Ipomopsis* pollinators and pollinator archetypes. Observing differences in floral traits are essential to understanding the underlying mechanisms that influence range shifts and predicting hybrid interactions.

I present my thesis in six chapters. Chapter one describes the natural history of *I. aggregata* and *I. tenuituba*. Chapter two explains the importance of studying natural hybrid zones, potential implications for conservation. Chapter three explores essential elements of phenotypic plasticity, genetic models describing the evolution of plasticity, and how research on this topic can be applied. The fourth chapter outlines hybrid zone theories, their importance and how relative hybrid fitness to parental species can be used to uncover hybrid zone limitations. Introductions to floral trait variation and my study systems, *I. aggregata* and *I. tenuituba*, are presented below in the remaining sections of the introduction. Chapter five details my hypothesis, methods, and the results of my experiments, and my final chapter includes a discussion and conclusion of my findings.
Chapter 1. Natural History:

**Scarlet Gilia and Slendertube Skyrocket**  
*Ipomopsis aggregata* and *Ipomopsis tenuituba*  
Phlox Family (Polemoniaceae)

*Ipomopsis* species do not go easily unnoticed. Their bright red and white flowers provide eye-catching displays that attract both pollinators and naturalists. The floral traits of species in the genus *Ipomopsis* differ more than the vegetative traits. Petal color in *Ipomopsis* var*ies* from red, pink, and yellow orange to white and lavender, while all species have similar growth habits and leaf size, shape and color. Habitats include low elevation piñon-juniper forests, foothills, montane woodlands, high mountain meadows, and rocky cliffs (Schneider 2015). Optimal growing conditions include little water, partial shade, and sandy soil (Schneider 2015). *Ipomopsis aggregata* range from southern British Columbia and Montana to Northern Mexico, while *I. tenuituba* are restricted to high elevation habitats in the same range (Fig. 1). *Ipomopsis* is part of the Phlox family (Polemoniaceae) native to the Americans, with the center of diversity in the Western United States.

**Phlox Family**

Polemoniaceae, the phlox family, which includes *Ipomopsis*, is comprised of about 400 species of herbs, shrubs, small trees, and vines (Fig. 2.). Members of this family are common desert, montane, or woodland wildflowers that form impressive displays during their brief blooming periods (Johnson 2008). There is not one distinctive morphological feature that identifies a member of the Phlox family, however, combinations of floral traits can be used to differentiate Polemoniaceae from other families of flowering plants. Five sepals, fused petals, and stamens that alternate with corolla lobes, and an ovary with three fused carpels are characteristics that often distinguish *Ipomopsis* from the rest of the
Phlox family (Plate 1). The three-part ovary, in combination with the other floral features, is key (Johnson 2008). Most Polemoniaceae species are annual plants that germinate, flower, and die within a twelve-month period. Others, however, are perennial and polycarpic (persisting more than two years and flowering multiple times during their lives) and a few are biennial (two-year lifespan) (Johnson 2008). *I. aggregata* and *I. tenuituba* follow an unique life history, different than most of the phlox family, but share common floral morphologies.

**Ipomopsis**

Andre Michaux first described the *Ipomopsis* genus in 1803 and Frederick Pursh first described what is today *Ipomopsis aggregata* in 1814 (Plate 2). (Schneider 2015). The specimen Pursh used was from a collection brought to him by Meriwether Lewis from what is today Idaho (Schneider 2015). Pursh first named the plant *Cantua aggregata* (Grant and Wilken 1986). Since then, the species has seen dozens of new classifications. The plant was named name *Gilia aggregata* in 1825 by Christian Konrad Sprengel (Grant and Wilken 1986) and that remained the name until 1956 when it was reclassified as *I. aggregata* (Schneider 2015). The first accounts of *Ipomopsis tenuituba* (Plate 3), were collected in Utah by Edward Palmer in 1877, but remained an undifferentiated species until 1913, when described as *Gilia tenuituba* (Schneider 2015). *Ipomopsis tenuituba* also was reclassified by Verne and Alva Grant in 1965 (Grant and Grant 1965). Much of this taxonomic confusion stems from the great variation in flower color and shape among populations. This variation and subsequent speciation may be driven by selection from different pollinators (Schneider 2015).
*Ipomopsis aggregata* and *I. tenuituba* have life histories unlike many wildflowers in the region and in the phlox family. *Ipomopsis* is a monocarpic perennial, with a taproot, soft woody base, and one to several erect stems (Fig. 3). (Campbell et al. 1997, Grant and Wilken 1988a). Each plant produces a rosette in its first year (Plate 4) and may live in a non-reproductive state for 2 to 12 years before producing one or many inflorescences (Fig. 3), and dying the following winter (Campbell et al. 1997). Herbivory and available resources can influence the number of years spent in the vegetative state (Campbell et al. 1991).

Vegetation is usually foul smelling with white woolly pubescence as seen in Plate 4 (Grant and Wilken 1986). Each inflorescence consists of a main stock, varying lateral stem abundance with one to three flowers and leafy bracts; flowers are often, but not always arranged along one side (Plate 3). Each flower forms an elongated tube, or corolla, from fused petals. Each corolla divides into five spreading corolla lobes at the tip (Schneider 2015). Petal color varies among scarlet, pink, orange, yellow, or white (Plate 5). Stamens are unequal in length with anthers that either extend out or are included in corolla (Schneider 2015). Yellow and sometimes white pollen are most common (Grant and Wilken 1986). Flowering stalks are often eaten by native wildlife and livestock, but plants can compensate for herbivory by sending up new shoots and branches (Fertig 2013).

Both species of *Ipomopsis* in this study begin flowering in late June and the flowering season extends into late summer, with the fruiting period extending into early fall. Seeds overwinter and germinate the following spring (Schneider 2015).
Pollinator-driven Speciation

*Ipomopsis aggregata* and *I. tenuituba* are frequently separated by extreme habitat variation from mountain meadows to steep rocky cliffs. However, extreme habitat shifts do not always create a complete isolating barrier, so there are frequently overlapping ranges or zones of sympatry (Grant and Wilken 1988b, Aldridge 2005). Hybrids occur frequently between adjacent populations of these species. The hybrid flowers appear intermediate in morphology and have a distinct, orange-red color (Plate 6) (Grant and Wilken 1988a). Both species and their hybrids are difficult to distinguish by vegetation alone (Campbell et al. 2003). Floral morphology contributes to reproductive isolation between the red-flowered *I. aggregata ssp. aggregata* and the pale-flowered *I. tenuituba ssp. tenuituba* in the Rocky Mountains of Colorado (CO) (Campbell et al. 1997, Meléndez-Ackerman and Campbell 1998). Hummingbirds (*Selasphorus platycercus* and *S. rufus*) prefer to visit the broader-tubed, red flowers of *I. aggregata*, whereas hawkmoths (*Hyles lineata*) select for slender-tubed flowers of *I. tenuituba*, leading to divergent selection on corolla width (Campbell et al. 1997, Bischoff et al. 2015). Although rare, in locations where these populations co-occur, foraging hummingbirds and hawkmoths visit both *I. aggregata* and *I. tenuituba* and often transition between them (Aldridge and Campbell 2007). Transitions between the plant species result in pollen transfer (Campbell et al. 1998) and interspecies pollinations generate as many seeds as conspecific pollinations (Campbell and Waser 2001). Thus, pollinator behavioral differences do influence hybridization.
**Ipomopsis aggregata**

Throughout history, taxonomists have named over 27 taxa of *I. aggregata* as individual species. Today, those 27 taxa are recognized as one of three species, each with 7 to 10 subspecies (Schneider 2015). *I. aggregata* grows throughout the western USA, usually below 2900 m elevation (Aldridge 2005). It blooms from late spring through summer and into early fall and flowers range from fiery red to red/orange and occasionally yellow (Plate 3) (Schneider 2015). One key distinguishing feature that separates *I. aggregata* from *I. tenuituba* is that *I. aggregata* produces stamens and anthers that do not extend beyond the opening of the corolla (Schneider 2015). In comparison to *I. tenuituba*, *I. aggregata* has a shorter tapered calyx and produces greater nectar volume and more concentrated nectar (Campbell et al. 1997). *I aggregata* produces nectar during the day to attract hummingbirds, *Selasphorus platycercus* and *S. rufus* (Campbell et al. 1997, Bischoff et al. 2015). *I. aggregata* flowers are generally odorless, but variation in volatile compounds occurs (Campbell et al. 1997). Although defined as hermaphroditic, *I. aggregata* has shown sex allocation in flowering months, with phenotypic gender reaching a proportion of 0.77 female components relative to male (Campbell 1992). More generally, *I. aggregata* flower shape differs from *I. tenuituba* by possessing a shorter and wider corolla, which can be associated with the beak shape of their hummingbird pollinators.

**Ipomopsis tenuituba**

*Ipomopsis tenuituba* populations tend to occur at higher elevations than *I. aggregata*, in subalpine fir and high elevation sagebrush communities (Schneider 2015). *Ipomopsis tenuituba* is less widely distributed, and typically grows at elevations above 3100 m (Grant 1959, Aldridge 2005). *Ipomopsis*


tenuituba has a pale pink to lavender, white, or more rarely a light purple flower (Plate 3) (Schneider 2015), with long, slender tubes; characteristics associated with hawkmoth pollination (Grant and Grant, 1965), although at some sites hummingbirds are the more common pollinator (Campbell et al. 1997, Aldridge 2005). Unlike I. aggregata, stamens and anthers usually extend beyond the opening of the corolla (Schneider 2015). Ipomopsis tenuituba generally has a longer, more slender calyx lobes with thinner petal lobes than I. aggregata and produces nectar in the early morning or late afternoon, with sweet smelling flower volatiles in the late evening to attract hawkmoths, Hyles lineata (Schneider 2015),

Another distinguishing feature of I. tenuituba is the production of the nitrogenous volatile organic compound (VOC) indole (Bischoff et al. 2014). Ipomopsis species emit large amounts of VOC's that include terpenoids, but indole is the main separating volatile between these two and the only nitrogenous VOC (Bischoff et al. 2014).

Indole is common to other hawkmoth-pollinated species such as: Hedychium flavum (Zingiberaceae), H. gardnerianum (Zingiberaceae), Angraecum sesquipedale (Orchidaceae), and Epidendrum cilare (Orchidacea) (Knudsen and Tollsten 1993; Levin et al. 2001) and is an antennal stimulant for H. lineata (Raguso et al. 1996). Indole is emitted just after sunset, during hawkmoths' active period, but not during the day (Bischoff et al. 2014). These results suggest that the synchronous timing of indole emissions with the crepuscular pattern of hawkmoth foraging activity might contribute to reproductive isolation (Bischoff et al. 2015). I. tenuituba largely has longer and thinner corollas compared to I. aggregata. Variation between these two species
and their floral traits, not only distinguish them, but also influence their isolation, speciation, and recombination as hybrids.
Chapter 2. Conservation Implications:

Conservation and the Changing World

Increased CO2 emissions associated with industrialization have already caused species extinctions, altered the species abundance, disturbed interspecific interactions, resulted in altitudinal and latitudinal shifts in geographic ranges of species, and altered phenology (Arrhenius 1896, Andrewartha and Birch 1954, MacArthur 1972, Parmesan 2006, Lenoir et al. 2008). Habitats in current landscapes are highly disjointed; species are being exposed to novel environmental stresses due to climate change, the expanding range of invasive species, and habitat degradation (Franks et al. 2007, Ackerly et al. 2010, Colautti and Barrett 2010, Ouborg et al. 2006, Willi et al. 2006). Although some plants have been found to adapt to these changing conditions, climate change can be a powerful selection factor, leading to the adaptive evolution of significant plant traits for an annual species in just a few generations (Franks et al. 2007).

Rapid climate change, together with habitat fragmentation, is leading to intense selective pressures and decreased genetic flow, respectively (Jump and Peñuelas, 2005). Climate change is also imposing multifaceted and opposing selective stresses on stable long-lived populations, which in turn constrain the extent and ecological benefits of phenotypic plasticity, modify species interactions, and decouple climate and local adaptation, leading to an increased susceptibility to extreme climatic events and to a greater risk of mortality of plants under the new climatic circumstances (Valladares and Niinemets 2008). Together, these results show that our current knowledge about the role of phenotypic plasticity in either shielding or intensifying the impact of global change on plant species and communities is not yet sufficient to predict future
biodiversity scenarios because of the complexity of the ecological and genetic limits to plasticity (Valladares et al. 2007). Thus, research on phenotypic plasticity and predicted climate change conditions, i.e. increased temperature and CO₂ concentrations, is needed to calculate the impact of global changes on herbaceous species similar to *Ipomopsis*.

**Phenotypic plasticity and global change**

Investigation of phenotypic plasticity plays an important role in illuminating the impact of global change on essential plant species and communities (Bawa and Dayanandan, 1998; Rehfeldt et al. 2001; Maron et al. 2004). The significance of phenotypic plasticity as a barrier against extinction has not been widely valued. In reality, the degree of species' extinction may have been grossly overestimated in many models of distribution shifts induced by climate change because plasticity in populations of threatened species is not considered (Thuiller et al. 2005). In a rapidly changing local and global environment, superficially adapted populations with low plasticity in naturally selected functionally important traits might be at a higher risk of extinction (Valladares et al. 2007). Evidence suggests that climate change should favor high levels of phenotypic plasticity in plants (Parmesan, 2006). However, climate change often involves simultaneous fluctuations in two or more abiotic and biotic factors, which may limit the extent to which plastic phenotypes can adapt to changing conditions. For example, *I. tenuituba* and *I. aggregata* express fewer and smaller flowers with decreased soil moisture; decreased precipitation associated with climate change could limit the ability to attract pollinators and decrease overall reproductive success (Campbell and Wendlandt 2013). Therefore, a wide range of imperfect solutions to the conflicting situations faced
by plants in changing and complex environments have been observed in nature. As shown by Valladares, (2007), three interacting species can lead to a broad range of adaptive values of plasticity, something that becomes more complicated by the spatial and temporal heterogeneity of abiotic factors.

The complexity that develops from the consideration of several species, abiotic factors, and their interactions can explain the co-existence of species with contrasting plasticities. Considering these interactions also raises the possibility that not all responses to global climate change are adaptive. It has been suggested that global climate change may alter phenotypic integration, resulting in branch abortion, cone production and changes in foliage dynamics induced by climatic variability in Scots pine (Martínez-Alonso et al. 2007, Valladares 2007). Thus, global change has the potential to induce differential plastic responses in co-occurring species and influence phenotypic integration that may in turn influence plasticity for certain traits.

Assessing climate change variation requires understanding of the diversity among plant species in the existing plant community and their growth strategies in response to changing resource availabilities (Valladares 2006). Since species with widespread geographic ranges have the potential to exhibit large intraspecific variation in physiology, morphology, and phenology, they may serve as good models for studying local and regional adaptation (Soolanayakanahally et al. 2009). Yet adaptation to future climate change could require the evolution of a number of different traits, which may be inhibited by correlations between said traits or their environmental triggers (Etterson and Shaw 2001). Therefore, it is essential to identify how plastic plant functional traits respond to global change in order to accurately predict species range shifts (Nicotra et al. 2010). By
identifying new species distributions, one can locate essential environments to protect species diversity in the changing landscape. Furthermore, it is crucial to fully understand the ecological consequences at species and ecosystem levels, keeping in mind that species with greater adaptive plasticity may be more likely to survive novel environmental conditions, since these changes generally occur too quickly to allow for an evolutionary or migratory response. Nevertheless, the extent to which phenotypic plasticity may assist survival under changing environmental conditions still remains largely unknown.

Climate change has already been shown to impose considerable selection for stress tolerance (Lenior et al. 2008, Parmesan 2006). Nonetheless, few studies have specifically tested whether species are adapting to novel conditions (Hoffmann and Willi 2008). Annuals and short-lived perennials are thought to have the capacity to adapt to changing conditions more easily than long-lived herbaceous and woody perennials because of shorter generation times (Anderson et al. 2011b). However, more studies are needed to determine this for certain. Plants are flowering earlier than ever previously recorded and have been found to be highly responsive to environmental conditions, such as earlier snow melt seen with *Boechera stricta* (Anderson et al. 2012). Plasticity in flowering time, as well as other floral traits, provides valuable insight into the evolutionary response to climate change.

Global climate change may also indirectly affect plants by affecting plant animal interactions, and, more generally, plant symbionts (Peñuelas et al. 2002, Hódar et al. 2004). Evidence suggests that climate change has been responsible for range shifts both away from the equator and to higher elevations of many species (Thuiller et al. 2005). However, not every species is affected by climate
change in the same way; range shifts have varied among species and have the potential to greatly modify interactions (Poorter and Lambers 1986, Sánchez-Gómez 2006, Thuiller et al. 2005, Valladares et al. 2007). This can lead to separation of pollinators and seed dispersers from their host plants, resulting in inbred populations and potential extinction. For example, *I. tenuituba* in Poverty Gulch, CO has only had visitations from their primary hawkmoth pollinator in 3 of the last 20 years, and climate change is thought to be the cause (Campbell et al. 1997).

Selection when these primary pollinators are present has a great impact on floral selection and plant fitness within this system, because fitness during these visitation years is significantly greater (Campbell et al. 1997). Seed production in years when hawkmoths are present has lead to almost double the fruit production compared to years without hawkmoths (Campbell et al. 1997). The presence of seed production in years without hawkmoths suggests that non-specific pollination does occur through incidental contact of other biota. By studying floral traits along a natural elevation and temporal gradient, I can explore how floral traits and their associated species' ranges are changing with world climate. Since the populations I am studying are associated with a natural hybrid zone, I can explore how the environment selects for novel traits along an elevational gradient.

As global climate changes, plants will not only have to adapt to an increase in ambient CO$_2$ levels but also to a change in the carbon-nitrogen levels in their tissues (Arnone et al. 1995). These changes may reduce the levels of secondary compounds and nutritional value of plant tissues and reduce food quality for herbivores, potentially causing an increase in herbivory and damage to the plant
An increase in herbivory can disrupt the mutualistic relationship many pollinators have with their host species, where adults pollinate and lay eggs on the host plant to consume and develop during early life stages (Arnone et al. 1995). A change in carbon-nitrogen levels may increase larval herbivory of the host plant; potentially until the death of the host plant. However if the host does not die, it may not produce flowers due to increased herbivory, and if it does, they could be consumed, denying the adult pollinator floral nectar. Reduced plant chemical defenses can also lead to an increase in overall herbivory and may not select for their mutualistic herbivores (Valladares et al. 2007). For example, *I. tenuituba* shares a symbiotic relationship with the hawkmoth, *Hyles lineata*. Adult hawkmoths are attracted to the flowers and oviposit on the leaves. Developing larvae feed on the plant, and develop into more moths that pollinate the plant. Climate change can have profound and multifaceted effects on species, due to fluctuations in abiotic factors that can greatly influence biotic factors, thereby amplifying levels of complexity.

**Habitat fragmentation**

In today’s highly disjointed landscapes, species are being exposed to novel environmental stresses due to climate change, changing ranges, and habitat degradation. Habitat fragmentation reduces total population numbers and average population size and increases geographic isolation (Ouborg et al. 2006). Population bottlenecks, genetic drift, inbreeding, and reduced gene flow have decreased genetic diversity in small fragmented populations, resulting in increased homozygosity, depressed fitness, and population extinction (Ouborg et al. 2006, Willi et al. 2006). Even long-lived wind-pollinated species have shown increased inbreeding, a reduction in diversity, and altered breeding systems as a
result of habitat fragmentation (Jump and Peñuelas, 2006). With increased habitat fragmentation, constrained genetic variation can limit adaptability of species to novel climates associated with climate change (Hoffmann and Willi 2008, Ouborg et al. 2006, Willi et al. 2006). Many studies have neglected the implication of fragmentation on the potential of species in the context of global climate change (Anderson et al. 2011a). Many species are not adapted to rapid environmental fluctuations associated with contemporary climate change, and may not have the plasticity for stress tolerance or dispersal mechanisms needed to withstand changing climate conditions or track preferred conditions (Massot et al. 2008).

We know very little about how habitat fragmentation affects adaptive evolution under projected climate change. Nevertheless, climate change can intensify inbreeding depression (Fox and Reed 2010); thus, inbred fragmented populations could show a reduced capacity to adapt to contemporary and changing environments relative to large unfragmented populations. Fragmented populations with decreased genetic diversity might lack variation for key ecological traits such as drought-tolerance, or pollination and dispersal, where artificially distributing individuals to suitable habitats may be necessary to prevent dramatic declines in species and genetic diversity (Damschen et al. 2006). Although these conservation practices have potential to prevent extinction with changing global climate, there is no guarantee that these techniques will be successful.

Recent studies suggest that under climate change, phenotypic plasticity is more likely to play a key role in survival than genetic diversity (Vitasse et al. 2010). Distinctive responses to climate can arise between populations throughout
a species’ distribution, but also between neighboring individuals in the same population (Jump and Peñuelas 2005). For example, increased summer drought may harm the regeneration of many species at their lower latitudinal and altitudinal distribution limits (Richter et al. 2012). Nevertheless, introducing more drought tolerant individuals to mitigate climate change might not necessarily be successful due to trade-offs between drought tolerance and growth plasticity. *Ipomopsis* flower number and size are significantly reduced under drought conditions (Campbell and Wendlandt 2013). These floral traits directly influence selection by pollinators and in turn the adaptive advantage of drought plasticity.

**High Altitude Mountain Plants**

The influence of global warming on terrestrial ecosystems has been shown to be greater in high mountain regions than in low latitude areas (Larcher et al. 2010). The projected rate of global warming in montane environments is expected to be three times greater than the global average rate of warming recorded during the 20th century (Gratani 2014). Dirnböck *et al.* (2011) hypothesized a rapid increase of plant species at risk of extinction. Additionally, species-specific reduction in fitness and diversity could change community dynamics by modifying competitive abilities. However, there is little information on adaptive potential in high altitude montane areas.

One of the predicted concerns of global climate change is the movement of plant species to higher elevations and latitudes, following the shift in habitat caused by climate (Jump and Peñuelas 2005). A drastic decrease in a species’ range or even extinction can be the consequence of migration towards higher altitudes. Rates and dynamics of these migration patterns will be highly
dependent on the habitat preferences of particular species and their functional
traits (Gratani 2014). Steep environmental gradients and patchy distribution of
habitats lead to small size and spatial isolation of populations and restrict gene
flow in *Poa alpine* (Stöcklin et al. 2009). Species with large adaptive capacities
may persist in their current areas if they have the ability to withstand
environmental changes (Linder et al. 2010). However, high altitude plants, such
as *Ipomopsis spp.*, are particularly vulnerable to extinction in the wake of
contemporary climate change.

**Plasticity and Taxonomic Issues**

Plasticity can also have a more applied purpose in systematics and
taxonomy by correcting inaccurate synonyms and classifications. Highly plastic
genotypes have often been considered different species (Whitman and Agrawal
2009). This is especially problematic when environmentally induced phenotypes
are confused as distinct species (Uvarov 1966, Whitman and Agrawal 2009). For
example, more than 27 divergent phenotypes of *Ipomopsis aggregata* were
previously assigned species status (Aldridge 2005). Inaccurate species
identification or failure to recognize phenotypic plasticity can obstruct basic
research.

**Forecasting Invasion**

Understanding the limitations of phenotypic plasticity has the potential to
aid in the prediction of invasive species advancement by identifying areas that are
particularly susceptible to invasion and by helping prioritize ecosystems and their
biota. Measuring phenotypic plasticity may help us forecast establishment and
spread of invasive species (Muth and Pigliucci 2007, Peacor et al. 2006), aid
conservation (Davis and Stamps 2004), and help us understand the
consequences of environmental disruption (Bradshaw and Holzapfel 2006, Hendry et al. 2008). Differential plastic responses among interacting species may alter ecosystem interactions (Visser et al. 2006). Studying plasticity, therefore, may improve environmental monitoring (Lee et al. 2006, Whitman and Agrawal 2009). For example, herbivore attack often induces a plastic defense response in plants, including the release of novel volatile compounds (Whitman and Agrawal 2009). Different plant taxa generally release different volatile blends. As such, scientists could monitor community stress levels by analyzing these volatile compounds in the local atmosphere (DeMoraes et al. 2004). Monitoring this response, and the resulting compounds can predict environmental health and susceptibility to invasion.

Conclusion

Despite the increasing number of studies on phenotypic plasticity, ecologists have found that demonstrating the costs and limits of plasticity is difficult (Valladares et al. 2007). The same could be said for phenotypically adaptive plasticity, which is often overlooked (Gianoli and González-Teuber, 2005; Ghalambor et al. 2007). In order to learn the constraints of plasticity, research on the relationships between gene expression and phenotype in conjunction with physiological studies, are needed to reveal trade-offs and functional limits (Van Kleunen and Fischer 2005). Phenotypic plasticity’s influence on the survival of plant species in the face of global climate change is still largely unknown.
3. **Phenotypic Plasticity:**

**Introduction**

Phenotypic plasticity is the ability of a given genotype to produce different physiological or morphological phenotypes in response to different environmental conditions (Bradshaw 1965, Callaway et al. 2003, Forsman 2015). A given plant genotype can experience different environmental conditions as a result of variation in the abiotic environment, variation in the presence or identity of neighbors, or variation in consumer pressure (Callaway et al. 2003). Ecologically important plasticity studies aim to test genotypes in a range of environments, based on natural variation, and focus on phenotypic traits that are important to function and fitness in those environments (Sultan 2004). Although the evolutionary aspects of phenotypic plasticity in plants have been extensively examined, we know little about the consequences of plasticity for ecological interactions in plant communities (Bradshaw 1965, Forsman 2015, Murren et al. 2015, Schlichting 1986, Sultan 1987).

As a rule, plants are highly plastic (Sultan 2004). Plants cannot move and must endure any and all conditions exerted by the environment, without the possibility of finding shelter or escaping enemies. Consequently, plants have evolved to be plastic in their physiology and development. Individuals within a species can vary greatly in size, chemical constituency, growth rate, allocation to different organs, and reproduction (Callaway et al. 2003). Plastic responses may be permanent once induced, relatively fixed for a given amount of time, or may be dynamic on a scale of hours, as in the case of the impact of light on photosynthetic chemistry or herbivore effects on defense chemistry (Baldwin 1999, Pearcy 1999). Plasticity allows an organism to adapt to varying
environments, potentially allowing for an ideal phenotype for every situation, rather than having to settle for one phenotype that may be highly adapted to one circumstance but average in most conditions (Pigliucci 2001). I measured floral and vegetative traits in *Ipomopsis spp.* to evaluate plasticity along an elevational cline.

**Phenotypic plasticity and the environment**

The environment has two roles in the evolutionary development of phenotypic plasticity. First, the environment establishes the fitness function, defined as the relationship between the phenotype of an individual and fitness, where fitness is how well a genotype can produce offspring (Whitman and Agrawal 2009, Murren et al. 2015). For example, the elevational gradient associated with *Ipomopsis spp.* at Poverty Gulch, Colorado, correlates roughly with soil moisture, which can significantly influence total flower number and size (Campbell and Wendlandt 2013). Second, the environment interacts with plant developmental processes and plays a role in determining phenotype (Scheiner 1993, Whitman and Agrawal 2009). For example, in *Ipomopsis spp.* specific leaf area and water use efficiency can vary depending on the environment during development (Campbell and Wendlandt 2013). The environment's influence on fitness function and development shapes how we interpret the environment's roles on phenotypic plasticity and how we refer to these impacts.

There are three terms that refer to the effects the environment has on phenotypic expression: phenotypic plasticity, reaction norm, and canalization. Phenotypic plasticity refers to the general effect of the environment has on tangible characteristic expression, while reaction norm refers to a specific genotype expressed across a range of environments (Scheiner 1993). Canalization
is the suppression of phenotypic variation due to genetic or environmental disturbances (Pélabon et al. 2004). For example, floral color can be canalized to attract pollinators in specialized mutualistic relationships. Canalization is often thought to be the opposite of plasticity; however, reaction norms can be canalized (Pélabon et al. 2004). Plasticity is not a general property of a genotype, but is specific to a trait or trait complex (Forsman 2015, Murren et al. 2015, Perkins and Jinks 1971). A given trait may be plastic in response to one environmental factor but not another (Scheiner 1993). For example, flower morphology maybe selected on by pollinators while SLA is selected on by UV radiation and water availability. Understanding the difference between reaction norms, canalization and plasticity is essential to understanding how species respond to environmental conditions.

**Stimuli and Cues**

Phenotypic plasticity can be initiated by either environmental stimuli or cues. Stimuli are common environmental factors such as temperature or oxygen level that directly disrupt homeostasis or development in non-adaptive ways and are often harmful. In contrast, cues are generally considered to be specific environmental signals that react to environmental change, stimulate adaptive plasticities, and tend to be beneficial stimuli (Whitman and Agrawal 2009). Although the cues that trigger phenotypic differences are environmental, the ability to respond to cues is genetically based and can evolve under natural selection (Bradshaw 1973). Genetic variation in phenotypic plasticity is an indicator of the potential for response to selection (Van Tienderen 1997). For example, knowing whether or not a species has high genetic variation in phenotypic plasticity may help us predict responses to changing environmental factors.
Genetic Variation and Phenotypic Plasticity

Phenotypic variation is believed to be the result of three factors: the genome, the effect of the environment on the genome (norm of reaction) and random accidents of development (Scheiner 1993, Zhang et al. 2012). The later two processes are often confused. Phenotypic plasticity entails environmentally mediated developmental change. Developmental accidents entail changes in developmental pathways due to random, internal events. These two independent processes are trait and environment specific (Schiener et al. 1993). However, some exceptions to this pattern have been shown in Drosophila melanogaster (Santiago et al. 1989, Scheiner 2014, Waddington 1961). Both environmentally mediated change and developmental accidents have the potential to increase and decrease fitness and therefore the potential to make plastic changes adaptive.

Adaptive Plasticity

Many examples of phenotypic plasticity are clearly adaptive, such as immune responses, antipredator defenses, acclimatization, diapause, life-history shifts, and dispersal (Lyytinen et al. 2004, Schmid-Hempel 2005, West-Eberhard 2003). However, the environment can influence phenotypes in complex ways, and it is often difficult to determine whether or not altered phenotypes are beneficial or adaptive (Pigliucci 2005, Van Kleunen and Fischer 2005, Whitman and Agrawal 2009). Plasticities are under contradictory selective pressures (Sih 2004) and carry numerous expenses and tradeoffs (DeWitt et al. 1998, Fordyce 2001, Whitman and Agrawal 2009). For example, plant species that are polycarpal annuals develop in different time periods and selective pressures, i.e. cold conditions in early growing season and hot conditions later in the growing season. Many traits may be changed by a single environmental factor, and not all
of these changes can be observed, including many complex physiological and environmental interactions and consequences (Agrawal 2005, Relyea 2004). To understand adaptive plasticity, one must consider benefits and costs of plastic phenotypes in several environments. Genetic and environmental correlations are, themselves, plastic to the environment (Pigliucci 2005). A particular plastic response may be highly advantageous in one season and detrimental in the next. A plastic response might, therefore, be evolutionarily favored, and in-turn maintained in a population, even if its expression produces great fitness benefits only once every 10 years (Whitman and Agrawal 2009). For example, fire can stimulate grasshoppers to change body color to black, which only affects populations periodically (Uvarov 1966). These selection pressures can result in enhanced resistance to certain stresses by selecting and saving stress tolerant plastic variation in the genome until the next selection pressure is present.

Genetic Basis of Plasticity

Genetically, plasticity likely results from differences in allelic expression across environments and to changes in interactions among loci; plasticity is not necessarily a function of heterozygosity (Scheiner 1993). The evolution of plasticity is modeled in three ways: overdominance, pleiotropy, and epistasis (Scheiner 1993) and these three models are not mutually exclusive. No model is perfect and each of the three models have limitations.

Genetic Models

Model 1: Overdominance. Plasticity is a function of homozygosity (Dobzhansky 1947, Gillespie and Turelli 1989, Lerner 1954). This model assumes that the amount of change in phenotypes across environments is a decreasing function of the number of heterozygous loci. This model is the most explicit with
regard to the genetic mechanism underlying plasticity (Scheiner 1993). Few data support this model. Most experimental studies have found no relationship between heterozygosity and amount of plasticity (Campbell et al. 2005). Although this model is not very applicable to experimental studies, the simplification of these mechanisms is important to clarify our understanding of the models that do work and their origin.

Model 2: Pleiotropy. Plasticity is a function of differential expression of the same gene in different environments (Forsman 2015, Scheiner 1993). This model assumes that the expression of an allele in one environment is potentially independent of its expression in a different environment. In an extreme version of this model, alleles are expressed in only one environment (Pigliucci 2003). This model is supported by many studies. Changes in the expression of single genes are well known. For example, heat shock genes become expressed at high temperatures (Pigliucci 2005, Scheiner 1993). By hypothesizing that the variation of phenotypic plasticity is a function of the same gene, one could account for variation in the environment to determine the range of plasticity for the mentioned gene. This may be over simplifying complex relationships among biotic and abiotic stimulants and their associated genotypes and phenotypes.

Model 3: Epistasis. This model states that plasticity is due to genes that determine the degree of response to environmental effects, which interact with genes that determine the average expression of the character (Forman 2015, Scheiner 1993). This model assumes that the trait mean and the trait plasticity are potentially independent characters (Scheiner 1993). Like pleiotropy, epistasis has received wide support (Forman 2015, Scheiner 1993). Specifically, with the single gene effect, the best examples are changes in the expression of regulatory
genes (Scheiner 1993). In contrast to Model 2, this model suggests that regulatory genes are the limiting factor to phenotypic expression. However, much of the support for this model has only looked at single regulatory genes, which is a great step towards revealing the mechanisms behind multiple gene interactions.

These three models have their own limitations and only two of three are supported by substantial evidence (Forman 2015, Scheiner 1993). However, these models may be too narrow because they only consider one genetic model, while models of the evolution of phenotypic plasticity need to account for both epistatic and pleiotropic effects (Campbell 2005, Campbell et al. 2005, Forman 2015, Scheiner 1993).

**Why is plasticity important?**

Phenotypic plasticity expands the gene-centered evolutionary theory (Whitman and Agrawal 2009). What was once accepted as background noise is now widely acknowledged as potentially adaptive. For the first half of the 20th century, scientists viewed evolution primarily as a mutational process that drove variation, while the environment selected phenotypes (Whitman and Agrawal 2009). However, this is not an accurate view because mutations are considerably rare and often deleterious (West-Eberhard 2003, West-Eberhard 2005). One environmental factor may alter the phenotypes of a whole population, selecting on perhaps thousands of individuals that have already expressed phenotypes based on existing environmental conditions, rather than on a single mutant individual.

Changing environments can potentially influence a diversity of traits that are not genetically linked. These could be rearranged in a novel climate, allowing for new phenotypes to occur every year. Recurrence of a novel phenotype among
large numbers of individuals that vary in numerous genetic, phenotypic, and environmental characteristics provides ideal conditions for selection (Whitman and Agrawal 2009). However, selection cannot act on a trait if that trait is not exposed to a stimulus or cue. By creating novel combinations of phenotypic traits, the environment produces new phenotypes for selection. This process is believed to lead to adaptive phenotypic plasticity that we see today, and even to the generation of new species (deJong 2005, Fordyce 2006, Schlichting 2004, West-Eberhard 2003).

Traditionally, the environment was thought to play a single role in evolutionary theory: selecting genetically produced variation. However, when considering phenotypic plasticity, the environment assumes a new role, creating phenotypic variation and selecting on that variation. This innovation elevates phenotypic plasticity to a dominant position in evolutionary theory (West-Eberhard 2003) Phenotypic plasticity also influences the debate on nature vs. nurture by affecting variation, because it combines the two concepts. Merging the influences genes and the environment have on phenotypes reduces the dichotomy of nurture and nature. Thus, genes and gene activities cannot be separated from direct environmental influence, and most traits represent a gene-by-environment interaction. This awareness elevates the function of the environment in gene expression and development, and the role of development in evolution, and is partially accountable for the recent surge in evolutionary developmental biology (Brakefield and French 2006, Sultan 2007).
When is plasticity favorable?

Plasticity is favorable in variable environments under two conditions. First, if the environmental variability is consistent across landscapes and reliable, individuals can express the appropriate phenotype in each environment. Secondly, plasticity is favorable when there are costs to having canalized phenotypes that do not fit in all environments of a species's range. Specialization is favored over plasticity when these circumstances are not met and when there are costs that limit species ranges (Dorn et al. 2000, Relyea 2002, Sultan 1995, Van Tienderen 1997). Because individual genotypes have the capacity for adaptive divergence, plasticity in plants should expand ecological ranges and reduce the impact of selection (Mazer and Schick 1991, Sultan 1995). Plasticity has been shown to increase fitness in multiple environments, expand niche breadth and increase range size (Pigliucci et al. 2006, Price et al. 2005, Schlichting 2004,). Plastic species can also potentially increase survival after natural disasters and avoid extinctions, because plasticity would allow species that have already survived through these experiences to be adapted for future catastrophes (Schlichting 2004). For example, Ipomopsis can survive drought conditions in years with little snowpack by having a variation in water-use efficiency influenced by the environment. Favorability can be also increased, because phenotypic plasticity creates a long-term strategy for evolutionary persistence. Plastic individuals may protect genetic diversity, allowing diversity to be stored, only to be expressed later, under specific conditions (Schlichting 2004, Suzuki and Nijhout 2006).
**Evolution of plasticity**

Potential pathways for the evolution of adaptive plasticity are through susceptibility, "non-functional" genetic material, and extreme environmental or biochemical conditions (Whitman and Agrawal 2009). In the first scenario, an environmental variable interrupts physiological homeostasis and development, creating new traits and new trait values, and rearranging phenotypes to produce novel trait combinations (Eshel and Matessi 1998). Most organisms contain large amounts of "non-functional" genetic material in their genomes. These genes are normally repressed via genetic canalization. Conversely, extreme environmental or biochemical conditions may disrupt gene repression, allowing the expression of hidden genes and novel phenotypes, while reducing expression of others (Whitman and Agrawal 2009). Although some changes are beneficial, most are probably not. Recurrence and selection would then adjust the regulation of gene expression and select for gene combinations that produce increased canalization or adaptive plasticities (Whitman and Agrawal 2009). Plasticities associated with abiotic factors such as elevational cline may have evolved this way.

Plasticity could also evolve when a previously existing plastic gene comes to serve a new function, potentially induced by a different cue, or a shift in its expression. For example, biochemical plasticity can evolve to produce morphological plasticity (Emlen et al. 2006). Likewise, plasticity could evolve after hybridization of two populations, each of which has evolved different fixed phenotypes (Whitman and Agrawal 2009). In this case, the hybrid population already had the ability to produce both phenotypes and only needed to connect differential reproduction to the environment. The evolution of new phenotypes does not require the evolution of new gene complexes, but only the repatterning
of already existing genetic material and epigenetic interaction (Emlen et al. 2006, Schlichting and Pigliucci 1998, Suzuki and Nijhout 2006). Subsequent evolutionary loss of flexibility can permanently canalize the trait. Consequently, a plastic trait can become subsumed into the genome as a canalized trait.

Studying plasticity is important for my research because one of the main questions I ask is to what extent is floral and vegetative trait variation based on plasticity or on genetic variation? By studying these relationships I can apply my results as preliminary data and begin to predict how these plants will be able to survive predicted climate change environments, increased CO2 concentrations and UV radiation. By comparing plastic variation between years, one can measure limitations of plasticity. However, this can be extremely time-consuming, considering that generation time can vary between 2 and 12 years. This system, on the other hand, has great potential for studying variation in plasticity at the population level from year to year, uncovering floral trait variation that might not be expressed every year, because only a fraction of each population blooms in any given year. One can gain a better understanding of plasticity by expanding my study of the 12 Poverty Gulch populations by measuring all individuals in the populations, rather than a subset. Outside the *Ipomopsis* system, a long-term study of plasticity of functional traits and/or conserved DNA, can expand the gene-centered evolutionary theory (Whitman and Agrawal 2009). This expansion is based on the idea the environment can drive variation and then select upon certain phenotypes, allowing non-linked traits to be rearranged in novel climate conditions, producing new phenotypes for selection.
4. Hybrid Zones:

Introduction to Hybrid Zones

Hybridization occurs when two separate species co-occur, are able to reproduce successfully. These offspring are known as hybrids. Not all hybrids are fertile and able to reproduce with each other or parental species. However, many species can reproduce viable offspring. Natural hybridization is fairly common in angiosperms, occurring in approximately 6-22% of all species (Campbell 2003). Hybrid zones can occur as result of a number of scenarios, which include a stable hybrid zone, the evolution of a new hybrid species, ongoing speciation or the breakdown of reproductive isolation after primary divergence (Barton and Hewitt 1985, Buggs 2007, Campbell and Wendlandt 2013, Campbell et al. 2008).

Hybrids have the potential to introduce new genetic variation into a population that can ease adaptation to changing abiotic conditions. Hybridization rates can vary between zones of sympatry because of variation in local abiotic and biotic factors (Aldridge and Campbell 2009, Campbell and Wendlandt 2013, Kirk et al. 2005). Comparing the fitness of hybrids to their parental species can unveil a great deal of information about the mechanisms of speciation. In order to speciate, divergent natural selection, or reduced fitness in intermediate phenotypes or hybrids must result in reproductive isolation (Campbell 2003).

The relative fitness of hybrids and their parental species has been used to develop models to predict and explain genetic differentiation across hybrid zones (Campbell 2003). Comparing zones of sympathy of closely related species has the potential to reveal complex isolating mechanisms, which includes isolation based on environmental heterogeneity in zones on sympathy (Aldridge and Campbell 2009). Natural hybridization between closely related species can represent
contact after a primary divergence event and uncover information about the
evolution of reproductive isolation and the underlying mechanisms driving
successful reproduction and isolation. However, differences in environmental
conditions between original and subsequent periods of divergence and may also
influence hybridization rate.

**How to Test Hybrid Zone Dynamics**

In order to test hybrid dynamics, one needs to assess three key elements:
hybrid fitness, fitness throughout life history, and fitness between parental and
hybrid generations. Fitness is a measure of how well a genotype can produce
offspring to the next generation. As stated previously, comparing hybrid fitness to
parental species is inherently important, as is measuring hybrid fitness in
multiple environments. By measuring fitness within environments, we can learn
whether fitness varies among populations or is intrinsically low. Temporal
variation in fitness and fitness between isolating and merging periods are also
important because short-term fitness mechanisms could vary from long-term
fitness. Evaluating hybrid fitness among classes can also bring insight to isolation
factors. Hybrid classes include F1 and F2 generation hybrids, as well as hybrids
with origins of different sex combinations of each parental species.

**How and why do hybrid zones occur?**

Hybridization in natural populations can have a variety of evolutionary
outcomes. These include, but are not limited to stabilizing hybrid zones, merging
of separate taxa into a single species, reinforcing of species differentiation, and
creating a new hybrid species (Campbell et al. 2008, Harrison and Harrison
The resulting outcome of hybridization largely depends on hybrid fitness compared to that of parental species (Campbell et al. 2008). There are three general hybrid zone structures that are accepted by theoretical and empirical evolutionary biologists: tension zones (Moore, 1977), bounded hybrid superiority zones (Harrison 1986) and mosaic zones (Buerkle and Rieseberg 2001, Howard 1986, Kirk et al. 2005). Models used to describe and predict hybrid zones are often based on hybrid fitness; however, other models are strongly influenced by environmental conditions (Anderson 1948, Arnold 1997, Endler 1977).

The tension zone model assumes that hybrids have an intrinsic reduced viability or sterility owing to defects caused by negative interactions between genes from parental species (Abbott and Brennan 2014, Barton and Hewitt 1985, Campbell et al. 2008, Kirk et al. 2005). Here, selection against hybrids independent from these genetic incompatibilities and hybrid zones are primarily limited to the clinal transition between parental species (Abbott and Brennan 2014, Campbell 2008). This model does not fit the Ipomopsis populations at Poverty Gulch, Colorado (Campbell 2008).

The bounded hybrid superiority model can be illustrated by a smooth transition between parental species, based on environmental conditions (Kirk et al. 2005). This model assumes a higher fitness in hybrid individuals in intermediate environments, but lower fitness in parental environments (Abbott and Brennan 2014). Species pairs that fit bounded hybrid superiority models tend to be found at ecotones, at the transition between environmentally distinct habitats (Abbott and Brennan 2014). The Ipomopsis populations used in my
experiments resemble the bounded hybrid superiority model, to some extent, because the hybrid zone exists in the ecotone where high montane meadows meet with rocky shale.

The mosaic hybrid zone model selects hybrids based on both environment and parental genotype frequencies. This mosaic model is represented by an assortment of distributed environments spread within the zone of sympatry (Abbott and Brennan 2014). This model does not represent the Ipomopsis system I studied, because the hybrid zone gradually varies between habitats and is not an assortment of distributed environments.

In some instances, species like hybridizing Ipomopsis, have different pollinators, but pollinator preference is not sufficient to prevent hybridization from occurring. However, most study systems that fit the bounded hybrid superiority models have generalist pollinators or are wind pollinated (Abbott and Brennan 2014). Across many species pairs, the most influential abiotic factors that select hybrids along altitudinal gradients are temperature and soil moisture. Temperature is particularly variable in altitudinal gradients due air’s ability to retain heat in thinning concentrations at increasing altitudes. Soil moisture also can vary considerably along altitudinal gradients in environments that include both flatlands and steep mountainous cliffs.

The Ipomopsis study model does not fall neatly into any of the three models previously described. Ipomopsis exhibits a smooth clinal change in morphological characteristics, but is not restricted by the hybrid superiority nor the tension zone models (Abbott and Brennan 2014). Classifying species pairs into one of these three models is exceedingly difficult because selection on
individual traits varies depending on plasticity induced by local environments and may obscure genetic variation based on trait measurements.

Hybrid Models

I am particularly interested in clinal hybrid zones because *Ipomopsis* plants exist along an elevational cline and some of their functional traits are spread out along this cline. Theoreticians have proposed at least five models to describe clinal hybrid zones: the hybrid disadvantage model, the environmental cline, hybrid advantage, neutral diffusion, and advancing wave (Moore 1977). The hybrid disadvantage model suggests that the cline favors the two parental forms due to natural selection, independent of environment and gene flow (Barton and Bengtsson 1986). More specifically, this model is based on the idea that F2 generation hybrids will exhibit reduced fitness because of genetic incompatibilities that stem from homozygous mutant loci that evolved in allopatric populations and have not been selected to function together (Campbell et al. 2008, Campbell et al. 1997, Buerkle and Rieseberg 2001). The environmental cline model proposes the selection of certain alleles at one limit of the cline and another allele at the opposing end of the cline (Campbell et al. 1997). The hybrid advantage model suggests hybrids have a selective advantage in area of sympatry (Buerkle and Rieseberg 2001, Campbell et al. 1997). The neutral diffusion model proposes the formation of a cline through the combination of neutral alleles after secondary parental contact (Campbell et al. 1997). Lastly, the advancing wave model suggests a selective advantage of one spreading parental species (Campbell et al. 1997). Fitting a hybrid system into one of theses models may over simplify the system and make assumptions.
However, applying models to these systems provides context for a comparison of hybrid zones.

**What Can Hybrid Models Teach Us?**

By studying hybridization rates, one can learn about trait distribution. In areas with adequate hybridization, zones of sympatry often contain individuals with characteristics intermediate between their parental species, frequently expressing a unimodal distribution of these characteristics (Aldridge and Campbell 2009). In areas of low hybridization, zones of sympathy often show a bimodal distribution, with individuals expressing parental type characteristics and few individuals with intermediate characteristics (Aldridge and Campbell 2009). These characteristics can be physiological, morphological and genetic (Aldridge and Campbell 2009). Understanding trait distributions is essential to understanding the environmental conditions that caused a species to diverge and the resulting ranges to remerge creating hybridization.

Exploring hybridization can also help predict responses to climate change. Modifications in environmental conditions resulting from climate change have the potential to alter ranges of related species to the point that the size of species overlap is either increased or reduced (Campbell and Wendlandt 2013). This change in range overlap could result in reproductive isolation because of increased or reduced viability of an immigrant species arriving in a novel habitat (Campbell and Wendlandt 2013). Climate change also has the potential to change environmental conditions in an area of sympathy, decreasing or increasing the rate of hybridization, by changing reproductive phenology, or altering pollinator behavior (Campbell and Wendlandt 2013). For example, climate change can
increase annual precipitation in an area of sympatry, which has been found to alter flower morphology in *Ipomopsis* species and therefore pollinator selection (Campbell and Wendlandt 2013). This would be an alteration in prezygotic reproductive isolation. Another way the changing climate can alter hybridization is if conditions changed the ability of hybrids to survive/reproduce, compared to parental species (Campbell and Wendlandt 2013). Reductions in viability or reproduction are mechanisms of postzygotic reproductive isolation (Campbell and Wendlandt 2013). By studying zones of sympathy one can learn hybridization limitations, which have the potential to predict species interactions with global climate change.

**Hybrid Movement**

Studying the movement of hybrids can help us understand species invasions, in addition to the causes and consequences of stability and the extinction of a parental species (Buggs 2007). Hybridization has brought many species to close to extinction (Allendorf and Lundquist 2003). Hybrid movement associated with clinal hybrid models has been found to be caused by climate change, where dominant alleles replace recessive ones (Buggs 2007). Hybrid movement, in populations that fit the hybrid advantage model, occurs when hybrids with greater fitness are not limited to the ecotone zone between parental species (Good et al. 2000). Hybrids can than expand their range towards both parental ranges. Asymmetrical or single direction range expansion may occur when one parental species's fitness is greater than the other. Movement in one direction may occur if repeated backcrossing of hybrids with parental species advances hybrids more closely related to said parental species. Movement in the hybrid disadvantage model or tension model, occurs when hybrids have a lower
fitness than their parents. These models predict that hybrid zones move based on population density gradients and asymmetry of hybridization in parental species.
Chapter 5. Hypotheses and Methods

Hypotheses

Exploring the geographical and temporal movement of hybrid zones provides practical evidence for conservation biologists investigating the dynamics of species invasions (Buggs 2007). Genetically based clines in floral traits can also help us to understand potential adaptation in responses to climate change. I re-measured floral traits in Ipomopsis in 12 populations along an elevational gradient in Poverty Gulch, Gunnison County, Colorado, which was first measured in 1991 and 1992, to answer two questions. First, How do present day elevational clines in corolla length, corolla width, and maximum anther position compare with those observed in 1991 and 1992 (Campbell et al. 1997) for populations of Ipomopsis located at this study site? Secondly, to what extent are elevational differences in corolla length and width due to phenotypic plasticity versus genetic differences? In accordance to my first question, total leaf hairs per leaf and standard leaf area (SLA) were also measured to compare vegetative trait clines with floral traits clines in 2015. I predicted that populations at higher elevations would have a greater average number of leaf hairs to help reflect UV rays, compared to lower elevation populations. Based on known patterns of specific leaf area, I predicted that populations of Ipomopsis would exhibit higher SLA in sites with wetter soils, and lower SLA in sites with drier soils. I hypothesized that selection on vegetative traits would differ from that of floral traits, because environmental conditions are not extremely varied among populations along the elevational cline. Yet, floral selection pressures are determined by three pollinators, where one of which is inconsistently present from year to year. By
investigating temporal floral trait variation, I can see if there have been cline-wide systematic changes or if trait variation is chaotic from year to year.

To explore the potential causes of temporal shifts in clines, I then used reciprocal transplant experiments to answer my second question: to what extent are elevational differences in corolla length and width due to phenotypic plasticity versus genetic differences? Based on known patterns of floral traits, I predicted that populations of *Ipomopsis* would exhibit relatively short and wide corollas in the species *I. aggregata* at lower elevations compared to higher elevations, while *I. tenuituba* should express long narrow corollas at higher elevation compared to lower (Campbell 2004).
Methods

Study System

Observations were collected from 12 populations (A-L) along a 3.7km transect mapped in Fig. 4. Three of these sites exist as part of a reciprocal transplant study, while the remaining nine are representative only of natural populations. This mountainous valley contains natural populations of *I. aggregata* at up to 2900 m in elevation and *I. tenuituba* at and above 3200 m, along with a natural hybrid zone between the parent ranges (Campbell et al. 1997). The wettest site is *I. aggregata* population L, our lowest elevation site at 2900 m (Campbell and Waser 2001). The driest site, I, is located in the middle of the hybrid zone, at 3050 m on a steep talus slope with little vegetation, population I (Campbell et al. 2004). Our highest elevation site is at *I. tenuituba* population A, near 3225 m. It has been 23 years since elevational cline data were collected from wild individuals, which is about 5 generations ago (Campbell 1997).

Floral Trait Measurements

To address my first question, at least two times per week, up to three open flowers from 10 plants were removed from each of the 12 populations, and the following traits were measured: corolla length, corolla width, anther position relative to corolla base (using calipers), and 48-hr nectar production and sugar concentration (using capillary tubes and a refractometer, as described in Campbell et al 1991). The majority of nectar measurements were collected on July 16 and 28, with the remaining measurements collected August 4. Corolla length, corolla width, and anther position were chosen to compare modern data with data from 1991 and 1992. All of these traits can influence pollination success (Campbell et al. 1997).
Vegetative Trait Measurements

To supplement floral trait measurements, I collected one mature basal leaf from each plant along my transects, at each of the 12 sites between July 16th and July 22nd. I then counted total leaf hairs per leaf and calculated specific leaf area (SLA). Specific leaf area is the one-sided area of a fresh leaf divided by its oven-dry mass. The area of each leaf was calculated by scanning the leaf using the program ImageJ. Number of leaf hairs of was counted from scans at 10x magnification. Samples were then oven-dried and weighed individually, and the mass was used to calculate SLA (as area (cm²) / dry mass (g)). These values were averaged for each site.

Experimental Gardens

In 2007, seeds produced by hand-pollinated crosses of *I. aggregata* and *I. tenuituba* (parental, F₁ reciprocal hybrids, and F₂ hybrids) were planted randomly at 10 cm intervals within 20 1×1 m blocks at each of the three experimental sites: *I. aggregata* population L, hybrid population I, and *I. tenuituba* population C. Blocks were placed along strips where there were no flowering *Ipomopsis* individuals from the natural population within 1 m to avoid encroachment of wild seeds. In 2008, 10 blocks of F₂ hybrids were planted at the lowest experimental site to increase sample size. For this study, F₂ hybrids provide high variation in trait values upon which selection can act. Each June, the surviving experimental plants are censused. Longest leaf length and number of leaves are measured for plants lacking inflorescences at that time. *Ipomopsis* exist as vegetative rosettes for a median of 5 years (at the lowest experimental site), bloom during a single season, set seed and die (Campbell 1997).
Data Analysis

I. Comparison of clines in 1992 vs. 2015

To evaluate question 1, an analysis of covariance (ANCOVA) was used to investigate floral traits data; corolla length, corolla width, and anther position, comparing fit to historical data, collected in 1992 with the data collected in 2015, with year as a factor and distance along the elevational transect as the continuous covariate variable (Fig. 5). For historical data, distance along the cline was estimated from data in Wu and Campbell (2005). In 2015, cline data were collected using a GPS.

II. Comparison of clines across traits in 2015

To compare clines in different traits, population means were scaled between zero and one by using:

\[
\frac{(Y - \text{min})}{(\text{max} - \text{min})},
\]

where \( Y \) is the population mean, and \( \text{min} \) and \( \text{max} \) are the overall minimum and maximum population means. These scaled values were then plotted against distance along the transect, for each trait in the direction that values increased with distance (Fig. 6.). The best fitting polynomial function to each cline was used to describe cline variation. A linear regression was used to assess the SLA cline.

III. Reciprocal Transplant Study

To evaluate question 2, a 3-factor analysis of variance (ANOVA) on floral traits was conducted, using site (e.g. \( I. \ aggregata, I. \ tenuituba, \) or hybrid sites), year, and plant genotype of origin (\( I. \ aggregata, I. \ tenuituba, \) F1 hybrid AT, the reciprocal F1 hybrid TA, or F2 hybrid) as categorical predictors. All interactions
were considered within the model, and were based on previous work that has shown responses of plants are expected to vary by genotype (*I. aggregata*, *I. tenuituba*, and cross-species hybrids) at a given site (Campbell, 2003; Campbell et al. 2010). A site effect would indicate plasticity, whereas an effect of genotype of origin would indicate a genetic effect. An interaction would indicate a genotype by environment interaction.

**IV. Trait Covariation**

To explore patterns of trait variation in different floral and vegetative forms among populations along the elevational cline, I calculated Pearson’s correlation coefficients between all measured traits in 2015. I also calculated an additional Pearson’s correlation coefficient between leaf surface area and total number of leaf hairs, rather than SLA, because surface area gives a better depiction of the leaf when comparing leaf hairs. SLA is a more informative vegetative trait for comparing to floral traits, because SLA can describe a plant’s relationship with the environment, and, more specifically, water and nutrient retention and protection from desiccation. Linear regressions were performed to compare traits. Only significant relationships (*P < 0.05*) were used to decrease redundancy of analysis of correlation and regression coefficients, and because estimates of regression coefficients are notoriously unreliable when relationships are weak. An analysis of bivariance was also calculated between all traits.

**Results**

**I. Comparison of clines in 1992 vs. 2015**

Both corolla length and corolla width showed a year by distance interaction in an ANCOVA (*P < 0.001* and *P < 0.005*). In a standard ANCOVA, with a factor of year and distance as a continuous variable, both traits were larger
on average in 2015 than in 1992 (Fig. 5). Corolla length was larger by 4.4 mm in 2015 (Fig. 5A, \( F_{1,242} = 13.54, P < 0.0001 \)), and corolla width was larger by 0.11 mm (Fig. 5B, \( F_{1,242} = 7.83, P < 0.005 \)). To evaluate whether increased flower size was systematic over years, I also examined data from 1991 (all populations), 1999 (populations I and L only) and 2013 (populations I and L only). Corolla length did not differ significantly between 1991 and 1992 (ANCOVA on population means, \( F_{1,20} = 0.09, P = 0.773 \)), and that trait appeared to increase systematically over years (note the consistent difference between open symbols and filled symbols in Fig. 5A). On the other hand, corolla width was variable from year to year and did not show a systematic difference (Fig. 5B). Corolla length and width varied between 2015 and 1992 differently than anther position.

The highest anther position showed a more complex pattern, in which the difference between 1992 and 2015 depended upon the location on the transect (year x distance interaction, \( F_{1,241} = 10.69, P < 0.001 \)). Anther position relative to corolla changed the most for *I. aggregata* populations lowest on the mountain (those with the highest values for distance in Fig. 5C), while changing little at the high *I. tenuituba* populations (those with the lowest values for distance in Fig. 5C). A strong clinal pattern with lower anther positions at the *I. aggregata* populations had been seen in 1992 (\( P = 0.0001 \) in model with distance effect nested within year), but no evident cline was seen in 2015 (\( P = 0.214 \)). Anther position did not differ significantly between 1991 and 1992, (\( F_{1,20} = 1.12, P = 0.302 \)). Thus the large values in 2015 are not just an anomaly.

**II. Comparison of clines across traits in 2015**

The slope for SLA was slightly lower than the slope for the floral traits: corolla length, width, and 48-hour nectar production (Fig. 6). However, this
vegetative trait did show a significant change with distance (linear regression, $F_{1,127} = 6.32, p<0.01$). Site K had the highest SLA value (202.774 cm²/g), while site F showed the lowest SLA value (145.273 cm²/g) (Table 1). The lowest elevation site, L, had the greatest 48-nectar production, 4.88 µL, while site B had the lowest 48hr nectar production with less than a microliter, 0.25 µL (Table 1).

**III. Reciprocal Transplant Study**

Since both corolla length and highest anther position showed changes between 1992 and 2015, I analyzed whether those traits show genetic or environmental variation. In the reciprocal transplant experiment, corolla length depended on both type of plant (*I. aggregata*, F1 hybrid with *I. aggregata* mother, F1 hybrid with *I. tenuituba* mother, F2, or *I. tenuituba*; $F_{4,152} = 7.22, P < 0.0001$) and on the site of planting ($F_{2,152} = 4.94, P < 0.001$). Those results indicate that both genetic and environmental factors influence the trait. Anther position also showed both genetic and environmental components of variation ($P = 0.012$ and $P = 0.035$ respectively). Although corolla length and anther position showed genetic and environmental influence, genetic factors were found to have a greater effect.

**IV. Floral Trait Covariation**

Of the six significant bivariate trait comparisons, the only pair with both a vegetative and floral trait was SLA and corolla length, which were significantly correlated (Fig. 7: $P < 0.050, r = -0.575, n = 12$). The only comparison of vegetative traits, leaf hairs and leaf surface area also correlated significantly (Fig. 7: $P < 0.01, r = 0.713, n = 12$). Corolla width and 24 hour nectar production correlated with the greatest significance and regression (Fig. 7: $P < 0.0001, r = 0.938$). Corolla length significantly correlated with more traits than any other,
with four significant correlations. Traits that covary with one another indicate that they may be limited by the same selection factor.
Chapter 6. Discussion and Conclusions

Discussion

I have demonstrated that corolla length in Poverty Gulch, Gunnison County, *Ipomopsis* has significant genetic and environmental bases. Since length has a genetic component and it changed systematically over years, that change is consistent with an evolutionary change, compared to the same populations from 23 years ago. However, alternative hypotheses need to be considered. Soil moisture has been shown to correlate with increased corolla length and may have influenced 2015 floral trait measurements (Campbell and Wendlandt 2013). Additional data from a future dry year would help to distinguish these possibilities.

Although a significant genetic difference has been found in both corolla length and width, corolla width did not establish system-wide variation when supplementary data from additional years were compared. Additionally, anther position varied significantly only when comparing lower elevational sites between 1992 and 2015. That change is consistent either with introgression of genes from the high elevational *I. tenuituba* sites or with environmental differences found just at low elevation.

Trait clines in this study were evaluated by ANCOVA. Yet, clines could be assessed in more depth by fitting clines to sigmoidal functions determined by the width, anther position and maximum slope, and comparing cline parameter values as in Derryberry et al. (2014). Sigmodial functions have the potential to better represent clinal changes through natural hybrid zones (Derryberry et al. 2014).
When considering snowmelt date as a means to infer soil moisture, one would speculate that a later snowmelt date would suggest higher soil moisture. Years with a later snowmelt date should have a systematic shift in higher soil moisture and in turn should have longer flowers across all populations. With this logic, longer flowers would be measured in 1999 (May 25\textsuperscript{th}), 1991 (May 22\textsuperscript{nd}), and 2013 (May 15\textsuperscript{th}) compared to snowmelt date in 2015 (May 7\textsuperscript{th}) and I did not find that occurring (Gothic Weather). There was no significant difference in corolla length when comparing flowers from 1991 and 1992, with snowmelt dates of May 3\textsuperscript{rd} and May 22\textsuperscript{nd} respectively (Gothic Weather). The changes in corolla length between years is less likely to correlate with water moisture or seasonal snowmelt date and more likely to represent genetic changes.

Two possible explanations for this genetic variability are based on (1) pollinator selection or (2) a moving hybrid zone. Pollinator selection as an explanation for the observed increased corolla length has the most supporting evidence, because of the systematic shift in corolla length across all populations along the elevational cline. Pollinators responsible for this selection include the hawkmoth (\textit{Hyles lineata}), the Rufus Hummingbird (\textit{Selasphorus rufus}), and the Broad-tailed Hummingbird (\textit{Selasphorus platycercus}). Both hummingbirds and hawkmoths prefer to visit flowers with longer corollas (Campbell et al. 1997, Campbell et al. 1991). Stating that pollinator selection is the driving force for this increase in corolla length is easy to state. However, there is a disconnect somewhere. Hummingbirds are found to pollinate flowers with longer corollas in this system, however in other systems humming bird pollinators are commonly attracted to shorter and wider corollas (Campbell et al. 1997), whereas hawkmoths are attracted to longer thinner corollas (Campbell et al. 1997). Which
would mean, that hawkmoths could be the driving factor leading to longer corolla length across the cline, because hawkmoths would fly from lower elevations to high elevations pollinating as they travel, increasing the corolla length along the cline. Yet, hawkmoths are scarce in this system and are not found from year to year (Campbell et al. 1997). Thus, the driving factor that is increasing corolla length along the cline is more complicated. Perhaps, longer corolla length is linked to greater fitness and these traits remain in the system throughout years when hawkmoths are not abundant. Another alternative would be humming birds are selecting for longer corollas, because they are associated with greater nectar production. Though, I found that corolla length has a negative correlation with increased nectar production. This evidence suggests that more experiments need to be conducted to explain why corolla lengths are increasing in this system. A potential implication of this data is that hummingbirds and hawkmoths with shorter mouthparts are being excluded from this mutualism. This might indicated that the mutualism between pollinators and Ipomopsis has become more specialized over the past 23 years and in turn is more vulnerable to falling apart, as environmental conditions change.

The alternative hypothesis, moving hybrid zone, suggests that longer corolla length genes are moving down the elevational cline through increased hybridization. Evidence for this hypothesis occurs when a trait remains the same in one section of a cline and has a significant difference in an adjacent section, shown in the anther position cline. This hypothesis is unlikely to apply to my system, because this pattern is shown in only one floral trait.

Hybrid zone movement in the present and recent past has been found to be a widespread occurrence (Buggs 2007). Patterns in natural zones are complex
and may be misleading because phenotypic characters can show introgression through hybrid zones in different directions (Buggs 2007). Similarly, *Ipomopsis* floral trait patterns are not systematically uniform and show evidence of possible introgression in different directions.

Although evidence for environmental and evolutionary pressures selecting for floral trait differences along a hybrid zone was found, hypotheses explaining these movements remain untested. The preliminary hypothesis of global warming has been suggested as a cause of the movement of hybrid zones (Buggs 2007). However, the need for more hybrid movement studies is essential. Evidence of common trends need to be found in multiple hybrid systems before climate change can be accredited for a causative factor of hybrid zone movements (Buggs 2007).

Implication for conservation of this study are particularly important because this study adds to the growing body of evidence for both plant and pollinator species ranges are changing with global climate change. Although, pollinator studies were not specifically conducted with is study, observations performed while collecting data suggest that *I. tenuituba* pollinators, *Hyles lineata*, are not abundant. I did not see a single hawkmoth during this field season. Additionally, populations of *I. tenuituba* were also considerably smaller than *I. aggregata*, where I had to strategically place my transect in a switch back formation to accommodate for smaller populations. Furthermore, *I. tenuituba* has a longer generation time, limiting the number of blooming individuals per year, and therefore the size of seed set production.

This system demonstrates evidence of range shifts in the past 23 years, but there are more data suggesting a lack of pollinators at higher elevations and a
growing specialized mutualism across all studied population that can eventually lead to local extinction. Implementing a hawkmoth conservation program to rear and release hawkmoths in the area may be necessary to keep populations of *Ipomopsis tenuituba* stable. Longer corolla length could be selecting for a small portion of the available hummingbird population, isolating resources only for pollinators with long enough mouthparts. Additional evidence is needed to support this claim. However, this evidence could be brought together by a number of future studies.

Potential experiments can be conducted to assess *Ipomopsis*-pollinator interactions, which include collecting pollinator observations, trait measurements, and abundance at all migratory locations. However the next logical experiment would be to determine if elevational differences in floral or vegetative traits are adaptive. Based on known patterns of floral traits, I predict that populations of *Ipomopsis* will exhibit relatively short and wide corollas in the species *I. aggregata* in lower elevations, while the species *I. tenuituba* expresses longer narrow corollas at higher elevation (Campbell 2004). If these patterns are adaptive, I predict that long, narrow flowers will increase fitness measured as seed production at high elevation while short, wide flowers will have higher fitness at low elevation. Adaptive patterns of this sort would have favored sharper clines over time, whereas increased hybridization (as proposed by Campbell et al. 2008) could have led to shallower clines. Sharper clines would suggest increased specialization of plant pollinator mutualism, favoring parental species rather than hybrids. To analyze variation in natural selection on floral traits, fitness of a plant will be estimated as its seed production. Relative fitness will be regressed on corolla length and corolla width using a model that also
includes the factor of site and the site-trait interactions to test if selection varies across space. To determine if plasticity is adaptive, I will see whether floral traits changed plasticity in the direction favored by selection for that site.
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## Appendix

### Table:

**Table 1. Nectar Volume and Specific Leaf Area**

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean Nectar (µL)</th>
<th>Standard Error</th>
<th>48 hr Nectar Production</th>
<th>Specific Leaf Area (cm²/g)</th>
<th>Standard Error</th>
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<td>A</td>
<td>0.88</td>
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<td>148.87</td>
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<td>0.13</td>
<td></td>
<td>153.67</td>
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Fig. 1. *Ipomopsis tenuituba* (A.) and *Ipomopsis aggregate* (B.) species range map. *Ipomopsis tenuituba* range is considerably smaller than *I. aggregata* and is often confined to high meadows and mountain tops (Kartesz 2015).
Fig. 2. Phlox family, *Polemoniaceae*, phylogenetic tree displays speciation to genus. The *Ipomopsis* clade is highlighted with a box (Johnson et al. 2008).
Fig. 3. *Ipomopsis aggregata* subsp. displaying variation in floral stock number, and size. Variation in floral stock is commonly induced by herbivory. Drawings by Linda Vorobik (Grant and Wilken 1986).
Poverty Gulch

Fig. 4. Poverty Gulch map displaying observational transects. Population sites are indicated by colored circle. Red circles indicate *Ipomopsis aggregata* populations, pink circles indicate hybrid *Ipomopsis* populations, and white circles indicate *Ipomopsis tenuituba* populations. Twelve sites are all situated along the north side of the trail.
Fig. 5. Temporal and spatial floral trait variation are presented in relation to distance from the highest population along the elevational cline. *Ipomopsis* genotypes are shown in data point color: white indicates *I. tenuituba* populations, orange indicates hybrid populations, and Red indicates *I. aggregata* populations. Means and standard errors across elevational clines are shown, where black lines represent data from 2015 and light lines represent data past data. ANCOVA shows a significant 2015 corolla length increase over values in 1992 ($F_{1,242} = 13.54$, $P < 0.0001$).
Fig. 6. Floral and Vegetative Clines from 2015 compares scaled population means between zero and one to compare traits by using: \((Y-\text{min})/(\text{max}-\text{min})\), where \(Y\) is the population mean. Circular points represent corolla traits, triangular points represent 48-hour nectar production, and rhomboidal points present specific leaf area. SLA showed a significant change with distance (linear regression, \(F_{1,127} = 6.32, p<0.01\)).
Fig. 7. Scatter plots showing trait mean values for pairs of traits with statistically significant correlations of *Ipomopsis* spp. Positive correlations are shown in the left column and negative correlations are shown in the right column.
Plate 3. *Ipomosis tenuituba* color ranges from pure white to lavender and pink shades. *I. tenuituba* corollas is usually longer and thinner than *I. aggregata.*

Photo credit: D. R. Campbell.
Plate 4. *Ipomopsis* spp. rosette. Species cannot be determined until flower emerges or genetic tests are performed. Notice white leaf hairs on both sides of leaves. Photo credit: G. R. Trujillo.
Plate 6. *Ipomopsis aggregate tenuituba hybrid* flagged showing elevational cline. Observed populations were measured along the right side of the trail. Photo credit: G. R. Trujillo.