

RUNNING TITLE: IPOMOPSIS HYBRID ZONE SPECIATION

Comparing clines in floral and vegetative traits along an elevation gradient
in an *Ipomopsis* hybrid zone

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Abstract

Gene flow in hybrid zones acts as a barrier to speciation, yet we still see cases in which separate hybridizing species are maintained. Selection for different floral traits at each end of a hybrid zone by pollinators and for different vegetative traits by environmental gradients are two possible sources of selection that could maintain different allele frequencies at different locations within a hybrid zone. In this study we measured five floral traits and three vegetative traits along an elevational gradient in an *Ipomopsis* hybrid zone and calculated their resultant clines to test predictions about selective forces that drive speciation and maintain species differences between *I. aggregata* populations at lower elevations and *I. tenuituba* populations at higher elevations within the hybrid zone. Corolla length increased with elevation, while corolla width, nectar production, relative redness and specific leaf area (SLA) decreased. The narrowest cline was in corolla length ($w=0.12$ km), followed by flower color, nectar production and corolla width ($w=0.43$, 0.97 , and 1.4 km, respectively) while the widest cline was in SLA ($w=1.7$ km). Cline centers were all similarly located along the hybrid zone with the exception of nectar production which was centered closer to the low elevation *I. aggregata* sites. The similarity of these clines' centers for all floral traits besides nectar production is consistent with a shift in the pollination regime approximately midway down the hybrid zone. Combined with past pollination research in this hybrid zone, the narrower clines in floral traits than in vegetative traits suggest that speciation is mainly driven by selection for longer, narrower, paler flowers at higher elevations by hawkmoths and shorter, wider, redder flowers at lower elevations by hummingbirds.

Introduction

Hybrid zones have been acknowledged as important systems for understanding evolutionary processes due to their large genetic variation across space (Whitham et al. 1999, Hewitt 1998, Arnold 1992). We know that because hybridization increases gene flow between populations with differing allele frequencies, it can act as a barrier to speciation (Vines et al. 2016). Nevertheless, we see examples in nature of separate species being maintained within hybrid zones, and our understanding of the mechanisms driving speciation in such hybrid zones remains understudied (Stankowski et al. 2015). Strong selection can maintain allele frequency differences (Vines et al. 2016), and in plant systems, there are two possible sources of that selection: pollinators and other aspects of the environment. For example, many biologists have suggested that pollination plays a major role in angiosperm speciation (Campbell 2003), and in hybrid zones, selection for certain floral traits by different pollinators could maintain different trait values at different locations. Environmental variation could also influence speciation within plant hybrid zones when factors such as soil moisture, temperature or nutrient availability select for different vegetative traits that confer selective advantages at different locations. One way we can approach questions about speciation in hybrid zones is by measuring traits of interest on individuals along a gradient and analyzing the resultant clines. Because the slope and position of clines tell us about the balance between dispersal and total selection at a locus for some particular trait (Vines et al. 2016), we can compare the slopes and positions of clines for chosen traits to test

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predictions about the mechanisms maintaining different trait values at different locations within the hybrid zone.

This study aims to test predictions about the mechanisms driving speciation in a naturally occurring *Ipomopsis* hybrid zone by measuring several clines in floral and vegetative traits along an elevation gradient. The study site is located at Poverty Gulch in Gunnison County, CO, and consists of parental plant species *I. aggregata* at lower elevation sites (2900 m) and its close relative *I. tenuituba* at higher elevation (3100 to 3250 m) sites, with hybrids varying in the expression of parent alleles at intermediate elevations. Individuals of *I. aggregata* tend to possess shorter, wider corolla tubes that are red in coloration, while individuals of *I. tenuituba* tend to possess longer, narrower corolla tubes that are pale pink to white in coloration. Campbell et al. (1997) have shown that hummingbirds, the primary pollinators of both *I. aggregata* and *I. tenuituba*, selectively visit flowers with wider corolla tubes and intense red coloration, while hawkmoths, the major pollinators of *I. tenuituba* in years that they are present, selectively visit flowers with narrower corolla tubes. When foraging under low light conditions, the hawkmoths also prefer white or red flowers (Bischoff et al. 2015). If there are different pollinators at the two ends of the hybrid zone, we expect to see a steep cline in floral traits due to strong selection for these different floral traits at each end of the hybrid zone. If the clines are driven by the same pollinators, we would also expect to see that the centers of the clines are in the same position. If there are strong habitat differences, we would expect steep clines in vegetative traits along the hybrid zone where *I. aggregata*-like vegetative morphology is selected for at lower elevations toward *I. aggregata* populations and *I. tenuituba*-like vegetative morphology is selected for at higher elevations toward *I. tenuituba* populations. However, the talus slopes between the *I. aggregata* and *I. tenuituba* populations on which the hybrids survive are drier than either parental site, followed by the *I. tenuituba* and the *I. aggregata* sites respectively (Campbell 2003). This non-linear pattern in water availability suggests that clines in vegetative traits may be less pronounced than for floral traits. If the same environmental characteristic selects on all vegetative traits, we would expect to see the centers of clines for vegetative traits to line up.

By quantifying clines in floral and vegetative traits in the *Ipomopsis* hybrid zone at Poverty Gulch, we aim to test predictions about the mechanisms driving speciation in this system. Streisfield and Kohn (2005) measured floral traits and genetic differentiation between populations in a *Mimulus* hybrid zone to test whether differences in flower color among populations could be attributed to natural selection or to drift followed by secondary contact. They found a strong genetic basis for flower color, and steep clines in flower color across populations at different geographical locations within the hybrid zone. Combined with far less pronounced patterns of genetic differentiation at neutral loci, this is evidence that current or recent natural selection on floral traits maintains flower color differences between locations within the *Mimulus* hybrid zone. Because, for heritable traits, stronger selection produces steeper clines (Vines et al. 2016), we can compare clines in floral and vegetative traits of interest to test our own predictions about selection within the *Ipomopsis* hybrid zone. If speciation in the *Ipomopsis* hybrid zone can be attributed to pollinator-driven divergent selection, clines in floral traits will be steeper than clines in vegetative traits, all else equal. This prediction does make the assumption that floral and vegetative traits each have similar genetic variance, as for a

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cline in a quantitative trait the width is expected to decrease not only with stronger selection, but also with higher additive genetic variance in the trait (Nurnberger et al. 1995). Such pollinator-driven divergent selection may sustain different allele frequencies for floral traits at each end of the hybrid zone, maintaining different floral morphologies in each *Ipomopsis* species despite the gene flow between them. Alternatively, if speciation can be largely attributed to environmentally-driven divergent selection, clines in vegetative traits will be steeper than clines in floral traits, all else equal. Such environmentally-driven divergent selection may sustain different allele frequencies for vegetative rather than floral traits at each end of the hybrid zone, maintaining different vegetative morphologies in each *Ipomopsis* species despite the gene flow between them.

Methods

Study System

Our study site is located at Poverty Gulch in Gunnison County, CO (Fig. 1). The system consists of twelve populations of *Ipomopsis aggregata*, *I. tenuituba*, or hybrid individuals up an elevation gradient ranging from 2900 m to 3250 m. The lowest two populations (populations K and L) are comprised solely of *I. aggregata* and occur in the floor of the valley at elevations of 2900 m and below. The highest three populations (populations A-C) are comprised solely of *I. tenuituba* and occur on the steep, rocky slopes from 3100 m to 3250 m. The intermediate populations (populations D-J) consist of *I. aggregata* and *I. tenuituba* hybrids located on the talus slopes between the pure *I. aggregata* and *I. tenuituba* populations (Campbell et al. 1997). In most years, both *I. aggregata* and *I. tenuituba* are pollinated by the hummingbirds *Selasphorus platycercus* and *S. rufus*; however, in the three years that they were abundant in the system between 1992 and 2002, the hawkmoth *Hyles lineata* was the major pollinator of *I. tenuituba* (Campbell 2003). At each of the twelve populations, one 30 m transect containing ten individuals was established by running a 30 m line through each population and marking the closest flowering individual to the line at every 3 m. Two transects were set up at population L, so there were a total of 130 plants. For those populations that were smaller than 30 m across (populations A, B, C, and F), a 10 m transect was instead established with individuals marked at every 1m.

Trait Measurements

Measurements of floral traits included corolla length, corolla width, longest stamen, flower color, and 48-hour nectar production for each plant. These traits were chosen because past research has suggested that they are related to pollinator visitation (Campbell et al. 1997). Furthermore, genetic variation has been demonstrated for all of these floral traits in *Ipomopsis* (Campbell 1996, Campbell and Aldridge 2006). The corolla length, corolla width and longest stamen were measured on three randomly chosen flowers on each plant and the average of these three measurements for each trait was taken to obtain a single value for each trait per plant. Corolla length was measured from the base of the calyx to the top of a random petal, corolla width was the inside measurement of the corolla tube opening, and the longest stamen was measured from the base of the calyx to the top of the tallest anther.

To measure flower color, we took the average of two measurements of the relative redness of two flowers from each plant using a reflectance spectrometer (OceanOptics)

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and SpectraSuite software. We chose to analyze reflectance for wavelengths only within the visible light spectrum, because *Ipomopsis* has been shown not to reflect in the UV (Melendez-Ackerman 1997). We defined relative redness as the proportion of reflectance within the red compared to the green $((R_{626-700})-(R_{476-550}))/((R_{410-700}))$, giving us a value between 0 and 1 with values closer to 1 representing “redder” flowers. Only male phase flowers were chosen for color analysis because the flowers’ colors begin to fade after switching to female phase.

To measure 48-hour nectar production, we excluded pollinators from a single unopened bud on each plant with a plastic straw. After 48 hours, we removed the calyx and used a 5 μ L, 32 mm capillary tube to extract nectar from each flower and measured the height of the nectar column. To obtain a measurement of nectar production in units of microliters/day, we used the formula $5x/64$, where x =mm of nectar produced in 48 hours.

Measurements of vegetative traits included specific leaf area (SLA), density of leaf hairs, and normalized difference index for a reflectance index wavelength of 705 nm (ND_{705}), which has been shown to be a proxy for chlorophyll concentration (Sims and Gamon 2002). We chose to measure SLA because past research has suggested that it differs between plants in wet and dry environments (Scheepens et. al 2010). For SLA measurements, we removed a single leaf near the base of each plant and calculated its area in ImageJ, then dried it for two hours at 60°C and measured its dry mass. SLA was then calculated by dividing a leaf’s area by its dry mass. These same leaves were used to calculate leaf hair density, which we chose to measure because it can also be an adaptation to dry conditions (Roy et al. 1999). Leaf hairs were manually counted on the scanned image of each leaf at 200% original image size, and leaf hair density was calculated by dividing the number of hairs on each leaf by that leaf’s area. To obtain an estimate of leaf chlorophyll content, we measured the reflectance of a single leaf toward the base of a given plant using a reflectance spectrometer (OceanOptics) and SpectraSuite software. We then calculated ND_{705} (Sims and Gamon 2002) as the reflectance at 750nm minus the reflectance at 705nm divided by the sum of the reflectance at 750 and 705nm $((R_{750}-R_{705})/(R_{750}+R_{705}))$, giving us a relative measure of leaf chlorophyll content across sites. Differences between *Ipomopsis* of different genetic backgrounds have been demonstrated for SLA and ND_{70} (Crowell 2012).

Data Analysis

Data analysis was conducted in R ver. 3.2.2 and SAS ver. 9.3. First, to test for clines in each trait separately, we performed separate linear regressions for each trait with distance along the cline as determined by GPS data as the explanatory variable and the average trait value in a population as the response variable. For these regressions, we used the function `lm` in R. For those traits that varied significantly with distance along the cline, we then scaled those trait values so that the steepness of their resultant clines could be compared. Traits were scaled using the equation $(Y-\min)/(\max-\min)$ where Y was the population mean for the trait in question, and $\min$ and $\max$ were the overall minimum and maximum population means. To compare the width of clines quantitatively, we fit cline models to scaled trait values. For each trait, we tried fits of the data to a cline with no tails, two tails, just a left tail, or just a right tail, following the equations in Derryberry et al. (2014). The model equations were fit using Procedure `nlin` in SAS. For each trait,

only the cline model with no tails performed well. This model has two parameters: the width of the cline and the center of the cline.

Results

Floral Traits

Corolla length, corolla width, 48-hour nectar production and flower color were found to vary significantly as a function of distance along the hybrid zone. Corolla length decreased with decreasing elevation ($F_{1,10}=12.56$, $P = 0.00532$, Fig. 2a), with individuals in pure *I. tenuituba* populations possessing longer corollas than those in pure *I. aggregata* populations. The cline in corolla length was also the narrowest of all clines, floral or vegetative ($w=0.12$ km, Fig. 3). Corolla width increased with decreasing elevation ($F_{1,10}=110.4$, $P < 0.0001$, Fig. 2b), with individuals in pure *I. tenuituba* populations possessing narrower corolla openings than those in pure *I. aggregata* populations. While the relationship between corolla width and distance was the strongest of all floral traits, the cline in corolla width was the widest of any of the floral traits ($w=1.4$ km, Fig. 3). Nectar volume increased with decreasing elevation ($F_{1,10}=10.59$, $P=0.00866$, Fig. 2c), with populations at lower elevations producing more nectar. The cline for 48-hour nectar production was the second widest after corolla width ($w=0.97$ km, Fig. 3). Relative redness increased with decreasing elevation ($F_{1,10}=36.12$, $P=0.00013$, Fig. 2d), with individuals in pure *I. aggregata* populations possessing relatively redder flowers than those in pure *I. tenuituba* populations. The cline for flower color was the second narrowest after corolla length ($w=0.43$, Fig. 3). Maximum stamen length showed a non-significant trend toward decreasing with decreasing elevation ($F_{1,10}=4.829$, $P=0.0527$, Fig. 2e). The centers of the clines for corolla length, corolla width and flower color were similarly located in the hybrid zone around 0.8 to 0.9 km (0.91, 0.75, and 0.84 km respectively), near populations H and I. The cline for 48-hour nectar production, however, had a center at 1.31 km between populations J and K.

Vegetative Traits

Neither leaf hair density nor ND_{705} varied significantly with distance along the hybrid zone, however SLA significantly increased with decreasing elevation ($F_{1,10}=33.09$, $P=0.0002$, Fig. 4a). The cline for SLA was the widest of all clines, both floral and vegetative ($w=1.7$ km, Fig. 5) with a center at 0.83 km similar to the centers of the clines in floral traits.

Discussion

All floral traits varied across space in the *Ipomopsis* hybrid zone except for maximum stamen length, which still showed a trend toward significance ($F_{1,10}=4.829$, $P=0.0527$). All of these floral traits vary genetically (Campbell 1996, Campbell and Aldridge 2006). The strong clines in length, width, nectar production and color between *I. aggregata* and *I. tenuituba* populations suggest that some selective force is acting upon these traits and maintaining allele frequency differences between the two species despite the gene flow occurring between them. Hummingbirds, the major pollinators of both species of *Ipomopsis*, prefer foraging from flowers with wider corollas typical of *I. aggregata*, but in the rare years that hawkmoths are present, they prefer foraging from flowers with narrower corollas typical of *I. tenuituba* (Campbell et al. 1997).

Additionally, hummingbirds prefer red flowers (Melendez-Ackerman and Campbell 1998) and hawkmoths prefer white flowers of *Ipomopsis* (Bischoff et al. 2015). Because hawkmoths are rare pollinators, however, most years fit the advancing wave model of directional selection in which *I. aggregata*-like traits are favored everywhere in the hybrid zone (Campbell et al. 1997). Given that floral traits vary across the hybrid zone, and that the major pollinators of each species prefer different floral traits, pollinators are likely a source of selection on floral traits that maintain different floral trait values at each end of the hybrid zone. One possible reason that maximum stamen length may not vary as much with elevation is that its strong genetic correlation with corolla length ($r = 0.9$, Campbell et al. 1998) runs counter to the selection imposed on this trait by hummingbirds. Hummingbirds export more pollen to other flowers when the anthers are exerted beyond the end of the corolla tube, which would tend to favor long stamens in *I. aggregata* populations (Campbell et al. 1998). But at the same time, the genetic correlation with corolla length would tend to restrict evolution of longer stamens, as *I. aggregata* has shorter corollas.

Despite the fact that soil moisture content varies among sites (Campbell 2003), the only vegetative trait that varied significantly across the hybrid zone was specific leaf area, which decreased with altitude ($F_{1,10}=33.09$, $P=0.0002$, Fig. 3a). Lower SLA indicates thicker or denser leaves. Scheepens et al. (2010) suggest that SLA decreasing with altitude could be a response to decreasing temperature, and they found that while SLA is plastic, it is at least partially genetically determined. Thicker or denser leaves could also help retain moisture at the drier *I. tenuituba* and hybrid sites (Campbell 2003).

When scaled, the clines in these floral and vegetative traits varied in both their widths and the positions of their centers. The cline centers for corolla length, width and color were similarly located around hybrid populations H and I ($c=0.91$, 0.75 , and 0.84 km respectively), and these coincident centers are consistent with the hypothesis that divergence in floral traits is driven by a shift in pollinators (Stankowski et al. 2015). One floral trait, nectar production, had a different cline center located around population J ($c=1.31$ km). This could be due to some different spatial pattern relating to nectar production in which selection does not favor a higher population mean until around population J, although this is difficult to understand because such a spatial pattern would presumably be driven by the same pollinators that drive selection on the other floral traits. Alternatively, there could have been environmental effects such as soil moisture that did not manifest in the expression of floral traits other than nectar production appearing around populations H and I. For example, Campbell (1996) found that in a drought year, nectar production in *I. aggregata* dropped to a mean of 0.81 uL/day, drastically lower than the mean 1.49 - 3.66 uL/day in the other four years of the study. Additionally, Carroll et al. (2001) found that experimentally induced drought in fireweed (*Epilobium angustifolium*) reduced nectar volume threefold, but only reduced flower size by 33%. If soil moisture was low in sites I and J then lack of water might have reduced nectar production there more so than other traits.

Interestingly, the center of the cline in SLA was similar to that of the floral traits near sites H and I ($c=0.83$ km) despite the fact that it is a vegetative trait that is not driven by spatial patterns in the pollinator environment as floral traits are. This could be a coincidence; that is, environmental conditions influencing SLA might be changing in a similar location to changes in the pollinator environment that influence floral traits.

Alternatively, it could be that loci associated with SLA are in linkage disequilibrium with loci associated with other floral traits. In this case, the genes for SLA would be found in association with the genes for floral traits more often than expected by chance, causing them to change similarly in space despite the fact that they are influenced by different selective forces. Nurnberger et al. (1995) suggest that linkage disequilibrium among loci affecting different traits is expected to increase phenotypic covariances toward the center of hybrid zones. While we did not test covariance among SLA and floral traits in our analysis, the cline centers are located toward the center of the hybrid zone near sites H and I (0.7-0.9 km).

The narrowest cline in floral traits, corolla length ($w=0.12$ km), was over ten times narrower than the widest cline in floral traits, corolla width ($w=1.4$ km). This dramatic difference in cline widths could be attributed to two explanations: (1) selection is stronger on length than on width, or (2) additive genetic variance is greater for length than for width. We have evidence for divergent selection in width and flower color, but not length; hummingbirds prefer wider (Campbell et al. 1997) and redder (Melendez-Ackerman and Campbell 1998) flowers while hawkmoths prefer narrower (Campbell et al. 1997) and whiter (Bischoff et al. 2015) flowers. In contrast, both hummingbirds and hawkmoths appear to select for longer flowers in *Ipomopsis* (Campbell et al. 1991, 1997). Streisfeld and Kohn (2005) also found divergence in corolla length, width and color across a *Mimulus* hybrid zone, with flower color having the greatest divergence of all measured floral traits. Knowing this, we would expect the clines in width and color to be steepest if the differences in steepness are due solely to differences in selection. It is possible, however, that the optimal corolla length under hawkmoth pollination is even longer than under hummingbird pollination, as those previous studies were too small to estimate optima precisely. An alternative explanation for why the cline in length might be narrower than the cline in width or color is that the phenotype of corolla length has more additive genetic variance. Greater additive genetic variance in corolla length would allow for the evolution of different corolla lengths at each end of the hybrid zone due to selection by pollinators to occur more rapidly than the evolution of different corolla widths or colors (Agrawal et al. 2002).

Of the five floral traits measured in the *Ipomopsis* hybrid zone, four varied significantly along the elevational gradient within the hybrid zone. This was only true for one of the three measured vegetative traits. The narrowest clines were in floral traits, specifically in corolla length, flower color, nectar production and corolla width ($w=0.12$, 0.43, 0.97, and 1.4 km, respectively) while the widest cline was in SLA ($w=1.7$ km). The clines for both corolla length and flower color were narrower than the cline in one species-specific molecular marker, which is presumed to be neutral ($w = 0.79$ for data from Wu and Campbell 2005), providing additional evidence that those floral clines are maintained by selection. Most molecular markers are not species-specific and show even less clinal structure (Aldridge and Campbell 2009). Combined with the results of Campbell et al. (1997) which found preferences for narrower flowers by hawkmoths and wider flowers by hummingbirds, as well as the preference for whiter flowers by hawkmoths (Bischoff et al. 2015) and redder flowers for hummingbirds (Melendez-Ackerman and Campbell 1998), the narrower clines in floral traits than in vegetative traits suggest that speciation is mainly driven by selection for longer, narrower, paler flowers at higher elevations by hawkmoths and shorter, wider, redder flowers at lower

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elevations by hummingbirds. Hawkmoths are rare participants in the pollination environment (Campbell et al. 1997), so if they were to eventually become absent in this system we would expect selection for *I. aggregata*-like floral morphology consistent with the advancing wave model suggested by Campbell et al. (1997). However, if hawkmoths continue to be present in the *Ipomopsis* hybrid zone, floral traits might diverge even further with time as pollinator mediated divergence enhances floral traits that mediate pollinator attraction (Van der Niet et al. 2014).

Literature Cited

- Agrawal, A. A., Conner, J. K., Johnson, M. T., & Wallsgröve, R. (2002). Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution*, 56(11), 2206-2213.
- Arnold, M. L. (1992). Natural hybridization as an evolutionary process. *Annual review of Ecology and Systematics*, 23, 237-261.
- Bischoff, M., Raguso, R. A., Jürgens, A., & Campbell, D. R. (2015). Context-dependent reproductive isolation mediated by floral scent and color. *Evolution*, 69(1), 1-13.
- Campbell, D. R. (1996). Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution*, 1442-1453.
- Campbell, D. R. (2004). Natural selection in *Ipomopsis* hybrid zones: implications for ecological speciation. *New Phytologist*, 161(1), 83-90.
- Campbell, D. R. & Aldridge, G. (2006). "Floral biology of hybrid zones." Chapter in Harder, LD and Barrett, S.C.H. (editors) "Ecology and Evolution of Flowers." Oxford University Press, Oxford, 326-345.
- Campbell, D. R., Waser, N. M., & Melendez-Ackerman, E. J. (1997). Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *American Naturalist*, 295-315.
- Campbell, D. R., Waser, N. M., Price, M. V., Lynch, E. A., & Mitchell, R. J. (1991). Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution*, 1458-1467.
- Campbell, D. R., Waser, N. M., & Wolf, P. G. (1998). Pollen transfer by natural hybrids and parental species in an *Ipomopsis* hybrid zone. *Evolution*, 1602-1611.
- Carroll, A. B., Pallardy, S. G., & Galen, C. (2001). Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany*, 88(3), 438-446.

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- Crowell, M. "Genetic and environmental variation in leaf traits and physiology of Ipomopsis." (2012):
http://www.rmbl.org/modules/Downloads/Publications/Crowell_2012_AIR.pdf.
- Derryberry, E. P., Derryberry, G. E., Maley, J. M., & Brumfield, R. T. (2014). HZAR: hybrid zone analysis using an R software package. *Molecular Ecology Resources*, 14(3), 652-663.
- Hewitt, G. M. (1988). Hybrid zones-natural laboratories for evolutionary studies. *Trends in Ecology & Evolution*, 3(7), 158-167.
- Melendez-Ackerman, E. J. (1997). Patterns of color and nectar variation across an Ipomopsis (Polemoniaceae) hybrid zone. *American Journal of Botany*, 41-47.
- Melendez-Ackerman, E., & Campbell, D. R. (1998). Adaptive significance of flower color and inter-trait correlations in an Ipomopsis hybrid zone. *Evolution*, 1293-1303.
- Nurnberger, B., Barton, N., MacCallum, C., Gilchrist, J., & Appleby, M. (1995). Natural selection on quantitative traits in the Bombina hybrid zone. *Evolution*, 1224-1238.
- Roy, B. A., Stanton, M. L., & Eppley, S. M. (1999). Effects of environmental stress on leaf hair density and consequences for selection. *Journal of Evolutionary Biology*, 12(6), 1089-1103.
- Scheepens, J. F., Frei, E. S., & Stöcklin, J. (2010). Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia*, 164(1), 141-150.
- Sims, D. A., & Gamon, J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote sensing of environment*, 81(2), 337-354.
- Stankowski, S., Sobel, J. M., & Streisfeld, M. A. (2015). The geography of divergence with gene flow facilitates multitrait adaptation and the evolution of pollinator isolation in Mimulus aurantiacus. *Evolution*, 69(12), 3054-3068.
- Streisfeld, M. A., & Kohn, J. R. (2005). Contrasting patterns of floral and molecular variation across a cline in Mimulus aurantiacus. *Evolution*, 59(12), 2548-2559.
- Van der Niet, T., Peakall, R., & Johnson, S. D. (2014). Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of botany*, 113(2), 199-212.
- Vines, T. H., Dalziel, A. C., Albert, A. Y., Veen, T., Schulte, P. M., & Schluter, D. (2016). Cline coupling and uncoupling in a stickleback hybrid zone. *Evolution*, 70(5), 1023-1038.

IPOMOPSIS HYBRID ZONE SPECIATION

- Whitham, T. G., Martinsen, G. D., Keim, P., Floate, K. D., Dungey, H. S., & Potts, B. M. (1999). Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. *Ecology*, 80(2), 416-428.
- Wu, C. A., & Campbell, D. R. (2005). Cytoplasmic and nuclear markers reveal contrasting patterns of spatial genetic structure in a natural *Ipomopsis* hybrid zone. *Molecular Ecology*, 14(3), 781-792.



Figure 1. Hybrid zone containing twelve *Ipomopsis* populations located at Poverty Gulch in Gunnison County, CO. The three highest-elevation sites (A-C) contain pure *I. tenuituba* populations, while the two lowest-elevation sites (K-L) contain pure *I. aggregata* populations. Intermediate populations (D-J) contain hybrids of the two species.

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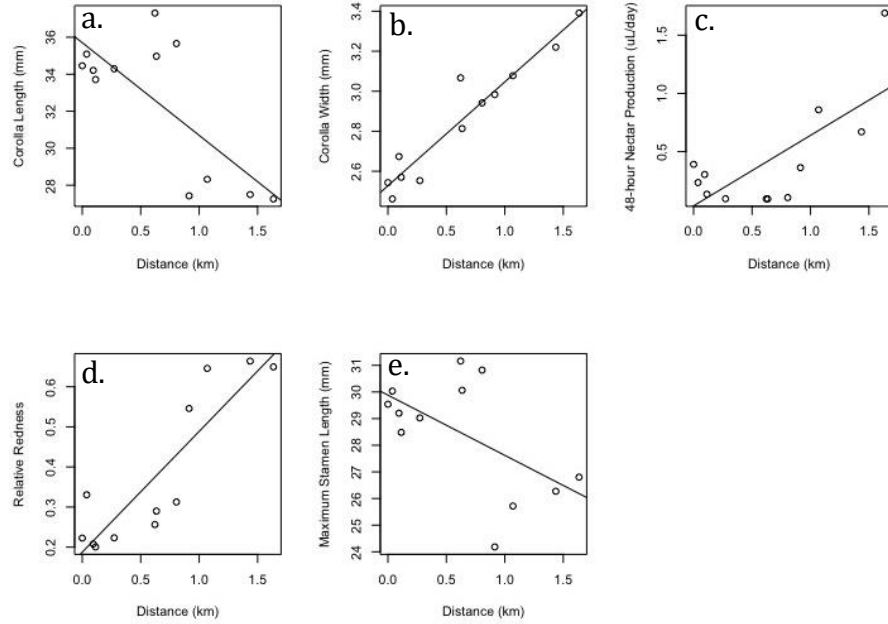


Figure 2. Variation in (a) corolla length, (b) corolla width, (c) 48-hour nectar production, (d) relative redness, and (e) maximum stamen length across the *Ipomopsis* hybrid zone. Trait values are scaled for comparison. Each point represents the scaled population mean for a single population A-L with *I. tenuituba* site A located at distance=0 km and *I. aggregata* site L located at distance=1.63 km. All traits besides maximum stamen length (e) showed significant variation with elevation.

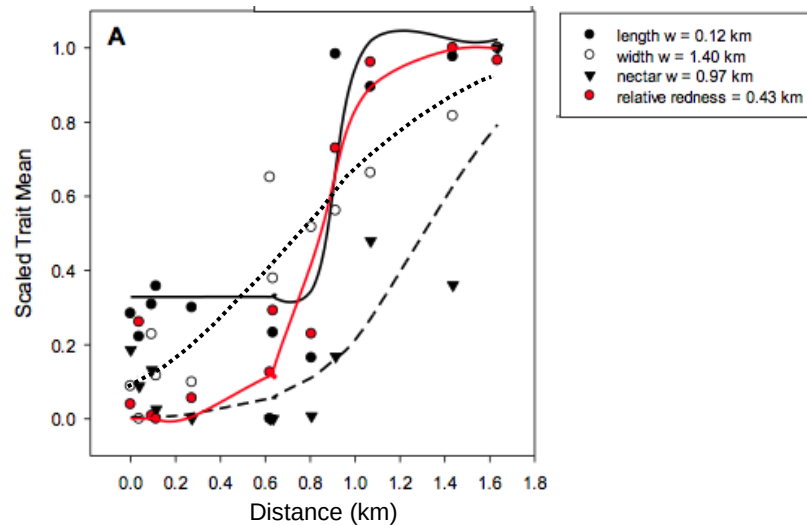


Figure 34. Clines in floral traits across an elevational gradient obtained by fitting the model: $Y=1/(1+e^{-4(X-cw)})$. Traits are scaled for comparison. Each point represents the scaled population mean for a single population A-L with *I. tenuituba* site A located at distance=0 km and *I. aggregata* site L located at distance=1.63 km. The values for corolla length have been scaled by subtracting the values from 1 so that they also increase with distance. The width of each cline is indicated in the legend (“w=”). Clines in floral traits all had similar centers ($c=0.91$, 0.75 , and 0.85 km for length, width, and color, respectively) except for 48-hour nectar production ($c=1.31$).

IPOMOPSIS HYBRID ZONE SPECIATION

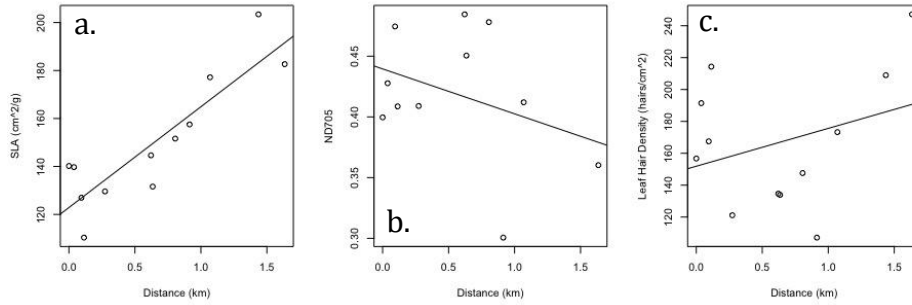


Figure 43. Variation in (a) specific leaf area (SLA), (b) normalized difference index for a reflectance index wavelength of 705 nm, and (c) leaf hair density across the *Ipomopsis* hybrid zone. Trait values are scaled for comparison. Each point represents the scaled population mean for a single population A-L with *I. tenuituba* site A located at distance=0 km and *I. aggregata* site L located at distance=1.63 km. SLA (a) was the only trait that varied significantly with elevation.

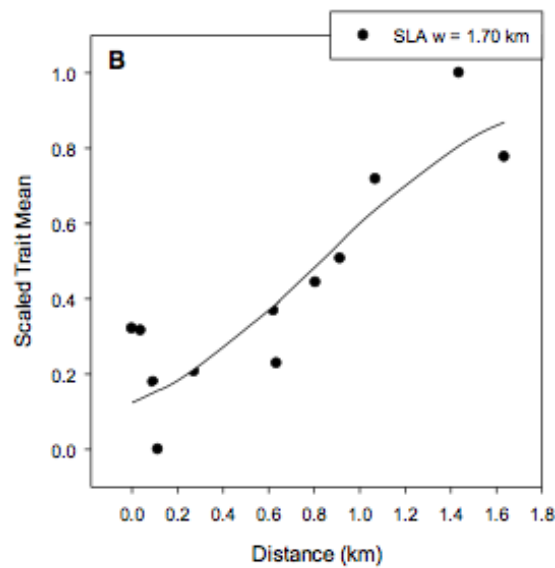


Figure 5. Cline in specific leaf area across and elevational gradient. Trait was scaled for comparison. Each point represents the scaled population mean for a single population A-L with site A located at distance=0 km and site L located at distance=1.63 km. The width of the cline is indicated in the legend (“w=”), and the center of the cline is located at 0.83 km.