

Investigating the impact of pollinator- and seed predator-mediated selection on floral traits in an *Ipomopsis aggregata* and *I. tenuituba* (Polemoniaceae) hybrid zone

Mentor: Dr. Diane Campbell

Student: Laura Barbour

Advanced Independent Research/REU

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Abstract

While the impacts of pollinator species on selection for floral traits in angiosperms are relatively well-studied, less is known about the contributions of herbivores towards selection on floral traits. We investigated the relationship among a variety of floral traits (including volatile emissions and morphological features) and oviposition by the seed predator fly *Delia* (Diptera: Anthomyiidae) in an *Ipomopsis* hybrid zone in Gunnison County, Colorado. We performed flower measurements on *Ipomopsis* individuals which had previously been planted at three elevations in a reciprocal transplant experiment at Poverty Gulch. Using a scent manipulation experiment near the Gothic town site, we also investigated the impacts of α - and β -pinene treatments on oviposition. We found no evidence for a correlation between *Delia* oviposition and any traits measured for Poverty Gulch flowers; however, other studies have suggested that oviposition may respond to increased corolla and sepal width. Single enantiomer pinene treatments [(+)- α , (-)- α , (+)- β , (-)- β] averaged significantly higher mean oviposition rates than our control. Additionally, our (+)- β treatment tended to have higher oviposition than our (-)- β enantiomer treatment, which is consistent with findings from 2011. When data from both years were combined, this difference was statistically significant. However, we also found our control to be significantly lower than our background oviposition rate. Our results indicate that treatments of single pinene enantiomers may increase attractiveness of *Ipomopsis aggregata* individuals to *Delia*. These results highlight the importance of incorporating seed predators and other herbivorous insects, as well as pollinator species, into investigations of floral trait adaptation and plant evolution.

Introduction

Plant hybrid zones offer a unique opportunity to study the multiple environmental, genetic, and physiological factors influencing speciation (Campbell 2003). If hybrid forms are

easily formed and are not strongly selected against, a breakdown of reproductive isolation can ensue (Campbell 2008, Servedio and Noor 2003, Wolf *et al.* 2001). Conversely, strong divergent selection favoring the two species can drive speciation or maintain differences between species (Campbell 2008). In postzygotic reproductive isolation, the intermediate hybrid phenotypes are less fit and the more specialized adaptations of the true species are favored, preserving more distinct species (Kimball *et al.* 2008, Servedio and Noor 2003). Prezygotic reproductive isolation prevents the formation of hybrids due to genetic, reproductive, or phenological incompatibilities between parent species (Servedio and Noor 2003). Hybrids may have reduced fitness due to genetic incompatibilities or may experience reduced fitness in the parent species' habitats simply because they are genetically less well-adapted to those particular environments (Campbell *et al.* 2008, Campbell and Waser 2000). In the first case, the hybrids would have reduced fitness regardless of environment (Campbell *et al.* 2008, Campbell and Waser 2000). In the second case, however, hybrid fitness is environment dependent and hybrid forms may perform as well or better than parent species in "hybrid" or intermediate habitat within the hybrid zone (Campbell *et al.* 2008, Kimball *et al.* 2008, and Campbell and Waser 2000). In angiosperms, selection in hybrid zones can act either on floral traits or on traits expressed during the vegetative stage (Campbell 2003). This study will focus on floral traits that may be under selection by seed predators as well as pollinators in an *Ipomopsis* hybrid zone (Irwin *et al.* 2003, Strauss *et al.* 1999, Brody 1992).

Ipomopsis aggregata (Polemoniaceae) and *I. tenuituba* are two closely-related species that have been shown to exhibit varying degrees of reproductive isolation when growing sympatrically (Campbell 2003, Grant and Wilken 1988). Instances of incomplete reproductive isolation result in hybrid zones (Campbell 2003). Both species occur in Gunnison County,

Colorado, and several hybrid zones are also present (Campbell 2003). At one such hybrid zone, in Poverty Gulch, *Ipomopsis aggregata aggregata* typically occurs at elevations below or about 2900 m and *I. tenuituba tenuituba* occurs at elevations greater than 3100 m, with hybrids found at intermediate elevations (Campbell 2003). Previous research suggests that seed predators and other herbivores, as well as pollinators, may be exerting selective pressures on the floral traits of these two parent species and their hybrids (Campbell *et al.* 2002, Brody 1992). In some instances, it is possible that pollinators and seed predators may exert opposing selective pressures on certain floral traits (Campbell *et al.* 2002, Brody 1992), and hence the selective outcome (in terms of floral trait-linked success) in a particular year would depend on a balance of the costs and benefits associated with pollinator attraction and avoidance of seed predation. The result would largely hinge on the prevalence, behavior, and types of pollinators and herbivores found in the hybrid zone during that reproductive season (Campbell *et al.* 2002).

Ipomopsis aggregata aggregata is preferentially pollinated by hummingbirds (*Selasphorus platycercus* and *S. rufus*) (Campbell 2003). Its corollas are red, and are relatively short and wide (Campbell 2003). These traits (wide red corollas) appear to increase pollination success by hummingbirds (Campbell 2008, Campbell 2003, Melendez-Ackerman and Campbell 1998, Campbell *et al.* 1996). *Ipomopsis tenuituba tenuituba* has pale pink-violet to white corollas which are longer and more slender than those of *I. aggregata* (Campbell 2003). In general, it depends more on hawkmoths (*Hyles lineata*) for pollination than on hummingbirds (Campbell 2003). However, hawkmoths are rare in Poverty Gulch, and hummingbirds are the main pollinators for the two parent species and their hybrids (Campbell 2003). When hawkmoths are present, it is possible that floral traits may experience increased disruptive selection (Campbell 2003).

Both species (and their hybrids) are also subject to seed predation by *Delia* (=Hylemya) ssp. (Diptera: Anthomyiidae), an herbivore with a highly specialized relationship to *Ipomopsis* and the closely related species *Polemonium foliosissimum* (Polemoniaceae) (Brody 1992). We collected data on floral traits (e.g. color, corolla width, nectar production rate, and volatile compounds) and *Delia* oviposition at the three Poverty Gulch sites to investigate the correlation between specific floral traits and seed predation rates. Additionally, we used an experimental approach (near the Gothic town site) to examine how treatments of the volatile compounds naturally produced by flowers affect oviposition rate.

The most abundant volatiles in emissions by flowers of these two species of *Ipomopsis* are pinenes, including α - and β -pinenes (and their +/- enantiomeric forms) (Irwin and Dorsett 2002, Bischoff, Juergens, and Campbell, unpub. data). Such floral scents can act as important signals mediating both plant-pollinator and plant-herbivore interactions (Raguso 2008). There is evidence that oviposition behavior in particular may be enantiomer-selective (Reisenman et al. 2010). Previous research has demonstrated that *Ipomopsis tenuituba* plants at Poverty Gulch are more successful than *I. aggregata* at avoiding seed predation by *Delia*, which oviposits on the interiors of the sepals (Campbell *et al.* 2002). Moreover, preliminary data suggest that *Delia* oviposition responds to pinenes (Feng 2011). The preference for *I. aggregata* may be related to morphological features of the flowers, as well as potentially pinene emissions (Campbell *et al.* 2002). In such a case, floral traits might experience differing selection pressures from seed predators and pollinators (Kephart *et al.* 2006, Campbell *et al.* 2002).

For instance, it has been previously suggested that floral traits such as increased sepal width and corolla width correlate with increased seed predation by *Delia*, which could explain the greater success of the narrower *I. tenuituba* flowers at avoiding seed predation (Campbell *et*

al. 2002). At the same time, narrower flowers may make *I. tenuituba* less attractive to hummingbird pollinators, a selective disadvantage, especially in years where hawkmoths are absent from Poverty Gulch (Campbell 2003). Concurrently, the varying α - to β -pinene ratios in the scent emissions of *I. aggregata* and *I. tenuituba* and their hybrids may also have differential impacts on both pollinators and seed predators, and ultimately reproductive success of the plants (Irwin and Dorsett 2002, Bischoff, Juergens, and Campbell, unpub. data). Our scent manipulation experiment was conducted to give insight into *Delia* responses to α - and β -pinene treatments applied on *I. aggregata* individuals. Combined data from the scent manipulation experiment and the flower trait measurements at Poverty Gulch enable us to address possible impacts of *Delia* seed predation on *Ipomopsis* individuals in this area, as well as make predictions about the joint selective forces that *Delia* and local pollinator species may be exerting on floral traits for *Ipomopsis* individuals.

Methods

Study System

Poverty Gulch, in Gunnison County, Colorado, is a naturally occurring *Ipomopsis aggregata* and *I. tenuituba* hybrid zone. At this site in 2007, Dr. Campbell and her lab set up a reciprocal transplant experiment in which seeds of known genetic background (pure parent species, F₁ reciprocal hybrids (AT and TA, where AT designates a hybrid with *I. aggregata* as the maternal parent and TA designates a hybrid with *I. tenuituba* as the maternal parent), and F₂ hybrids were planted at three sites along an elevational gradient. Twenty meter-squared blocks (with seeds from different genetic families planted randomly at 10 cm intervals using a grid system) were planted at each site: a lower-elevation site where *I. aggregata* occurs naturally (9,500 ft), a naturally occurring hybrid site of intermediate elevation (10,000 ft), and a higher-elevation *I. tenuituba* site (10,600 ft). Blocks were established along strips where there were not

naturally-occurring flowering *Ipomopsis* plants within 1 m, to ensure that any *Ipomopsis* growth within the blocks the next year was from experimental seeds. In 2008, another 10 blocks of F₂ hybrids were planted at the low elevation site to supplement the sample size. As the individuals planted at these sites are from multiple genetic backgrounds, they display a wide range of floral phenotypes, which enabled us to investigate correlations among floral traits and *Delia* oviposition over a broad range of floral variation.

Floral Trait Measurements

We measured floral traits for all surviving experimental plants which developed inflorescences this year (35 plants), using female phase flowers collected two to three times per week. Traits measured upon these flowers included corolla width and sepal width (to the nearest 0.1mm using calipers). Corolla width and sepal width are both traits which have been previously found to correlate to *Delia* oviposition (Campbell *et al.* 2002, Zimmerman and Brody 1998). We measured up to five female phase flowers per plant when possible, and averaged these data for each trait by individual so that we had one average measure of each trait for each plant.

Additionally, we measured nectar production after 48 hrs (using microcapillary tubes) because it is possible that nectar production correlates with overall scent emission, which may impact *Delia* oviposition. We covered fully elongated buds on up to two flowers per plant at the Poverty Gulch sites to prevent pollinator access and then returned and measured nectar production after approximately 48 hrs. Nectar data was also averaged by plant before analysis.

We also censused presence and number of *Delia* eggs in relation to the number of fully open flowers per plant each time we visited a site. At the end of the field season, the total number of eggs observed for each plant over the summer was calculated and divided by the total number of flowers examined on that plant, to give a ratio of total eggs per flower for each flowering plant at the Poverty Gulch sites.

We measured flower color spectrum (using a reflectance spectrometer and SpectraSuite software) on up to two male phase flowers per plant, as flower color may impact visitation by herbivores, as well as pollinators (Brody1997). We focused our calculations on reflectance in the visible wavelengths (401 to 700 nm) as *Ipomopsis* does not reflect in the UV (Melendez-Ackerman 1997). We calculated reflectance in the blue spectrum by summing the reflectance data from 401 to 475 nm and dividing by the total reflectance from 401 to 700 nm. Similarly, we calculated reflectance in the green, yellow, and red portions of the spectrum by summing reflectance data from 476 to 550 nm, 551 to 625 nm, and 626 to 700 nm, respectively, and dividing each by the total reflectance from 401 to 700 nm. We then subtracted reflectance in the green wavelengths from reflectance in the red wavelengths to give a measure of the redness of each flower (R-G). We also subtracted reflectance in the blue from reflectance in the yellow (Y-B) to give another value which approximates the inverse of the blueness for each flower. R-G values for each *Ipomopsis* individual were then averaged, as were Y-B values for each individual.

Throughout the summer we also gathered data on floral fragrance during day and night (such as pinene emissions) from *Ipomopsis* individuals at Poverty Gulch. Unscented oven bags were used to cover a flower and then a pump was attached to run the headspace over the trapping agents to collect volatile emissions. Floral scent was collected on two different trapping agents, Tenax TA[®] and SuperQ[®]. These data will be analyzed later using gas chromatography-mass spectrometry (GCMS). Tenax TA[®] samples will be analyzed with thermal desorption whereas SuperQ[®] samples were immediately eluted with pure hexane and will be analyzed with traditional solvent-based GCMS methods. Hexane injection will allow identification of the different pinene enantiomers. The data will be available later in the fall. Once analyzed, these

data will enable us to investigate correlations among specific floral traits (such as corolla width or nectar production) and volatile composition.

Scent Manipulation Experiment

In order to determine the impacts of pinene emissions on seed predators (*Delia*), we performed a scent manipulation experiment on *Ipomopsis aggregata* near Maxwell meadow south of RMBL (Gunnison County, Colorado). We applied 6 treatments and a control: dilutions of the (+)- α , (-)- α , (+)- β , and (-)- β -pinene enantiomers in scent-free mineral oil, as well as a 1:1 ratio of α - and β -pinenes in a racemic mixture (equal parts of each enantiomeric form) in mineral oil, and a 40:1 ratio of α - to β -pinenes in a racemic mixture in mineral oil applied at the plant level. The CAS (Chemical Abstract Service) number for each enantiomer was 7785-70-8 for (+)- α , 7785-26-4 for (-)- α , 19902-08-0 for (+)- β , and 18172-67-3 for (-)- β . The 1:1 ratio mimics the natural α - to β -pinene ratio in *Polemonium* volatile emissions (which is also subject to seed predation by *Delia*), and the 40:1 ratio of α - to β -pinene mimics the natural daytime ratio of the two pinenes in *Ipomopsis aggregata* (Feng 2011, Irwin and Dorsett 2002, Bischoff, Juergens, and Campbell, unpublished data). Mineral oil was our control. We also attempted to approximate the natural daytime emission rates of *Ipomopsis aggregata* (4794.5 ng/hr for α -pinenes and 119.5 ng/hr for β -pinenes) in our treatments (Bischoff, Juergens, and Campbell, unpublished data).

Our emitters were clean 1.5 ml microcentrifuge tubes with unscented medical cotton rolls cut into 5mm lengths inserted to a few mm below the top of the tubes. Our single enantiomer α -pinene treatments contained 350 μ l (+)- α or (-)- α -pinene in 20 ml mineral oil. Our single enantiomer β -pinene treatments consisted of 10 μ l (+)- β or (-)- β -pinene in 25 ml mineral oil. Our 40:1 ratio treatment had 400 μ l α -pinene [200 μ l (+)- α and 200 μ l (-)- α] and 10 μ l β -pinene

[5 μ l (+)- β and 5 μ l (-)- β] in 23 ml oil. The 1:1 treatment had 180 μ l α -pinene [90 μ l (+)- α and 90 μ l (-)- α] and 180 μ l β -pinene [90 μ l (+)- β and 90 μ l (-)- β] in 20 ml oil. 300 μ l of the appropriate treatment solution was pipetted onto the cotton in the microcentrifuge tubes. 300 μ l of mineral oil was pipetted onto the cotton in the control tubes. The tubes were closed and refrigerated overnight in separate microcentrifuge tube racks by treatment. After each day of treatment the cotton was replaced and the treatments re-pipetted. We stored the microcentrifuge tubes separately by treatment and reused them throughout the experiment, taking care to consistently use the same tubes for the same treatment.

I flagged 84 *Ipomopsis aggregata* individuals using rolled up and numbered white flags and randomly assigned a treatment to each individual. We applied treatments to plants at least 1 m apart (to minimize any cross-contamination from the effects of different treatments). We used wooden skewers near the base of each plant to support our emitters, which were attached to the skewers with twist ties at flower height on the plant. Feng (2011) found that the optimal time for *Delia* oviposition lasted 4-5 days, from the point at which the corollas of buds exceeded the length of the sepals to the point at which flowers were fully open. I therefore applied treatments (replacing the treatments in the emitters each morning) for 4 consecutive days and censused male phase flowers on each plant for *Delia* eggs on the morning of the 5th day. As newly opened flowers only remain in male phase for approximately 2 days, we could be sure that the flowers we were censusing did not contain eggs that were laid before the beginning of our treatment applications. We also determined the background oviposition rate each week from male phase flowers censused on 12 haphazardly selected plants spaced at least 1 m apart at the same site.

We repeated this protocol for 4 weeks in July. Treatments were put out each morning between 8 and 9:30 am and taken in between 3:30 and 5:30 pm each afternoon, in an attempt to

cover the times of maximum activity by *Delia*. All egg censuses were conducted between 7:30 and 9:30 am on the fifth day following our 4-day application of treatments to prevent additional oviposition on the plants before our census. For the first 2 weeks of the experiment, we reflagged plants between weeks and re-randomized treatment assignments. For the final 2 weeks we did not reflag plants, and we applied the same treatment to each plant for both weeks in an attempt to maximize the number of flowers censused per plant. Between the 3rd and 4th week of the experiment, we also collected scent samples for each treatment, the control, the emitter (cotton in microcentrifuge tube with no liquid added), and background air at the experimental site. We followed a similar protocol to the scent sampling at Poverty Gulch. We sampled in the morning (9:05 to 10:05 am) and in the afternoon (3-4 pm) to capture and verify emissions by our treatments and control over the range of time that our treatments were typically in the field. These data will also be analyzed using GCMS and should be available later in the fall.

Analysis

For the floral trait measurements from the Poverty Gulch sites we examined correlations between each floral trait and the totaled eggs per flower for each individual as our dependent variable. We excluded from the analysis individuals whose egg counts were based on 5 or less flowers. Oviposition rate was also compared among the three sites using a One-Way ANOVA. For the scent manipulation experiment, we analyzed data for each group of plants separately (week 1, week 2, and weeks 3-4) and then combined data by treatment across all weeks. In the analysis for combined data for all weeks, we excluded individuals from which we had collected data from less than 4 flowers. We used an ANOVA to test for differences in oviposition rate (eggs per flower censused) among treatment types. This ANOVA was supplemented by a priori contrasts between the two enantiomers of α -pinene, between the two enantiomers of β -pinene, between the averages for α -pinene and β -pinene, between the average of all four pinenes and the

control, and between the control and the background rate. We also ran an ANOVA incorporating our combined 4 week data for this summer with data from two weeks of a similar scent manipulation experiment that was conducted last summer (Feng 2011) where all four single pinene enantiomers were included as treatments [(+)- α , (-)- α , (+)- β , and (-)- β], testing for year x treatment interaction as well as differences in *Delia* responses among treatments. All data were analyzed using SAS statistical software.

Results

Floral Trait Data

None of the floral traits correlated significantly with oviposition rate (eggs per flower). The highest correlation measured ($r = 0.24$, $N = 28$, $p = 0.21$) was with corolla width. Site did have a significant impact on oviposition ($F_{2,26}=7.59$, $p=0.0025$). Oviposition at the hybrid site was lowest (0.1227 ± 0.0307 , mean $\pm$ standard error). Oviposition rate at the *I. aggregata* site averaged 0.475 ± 0.083 , and at the *I. tenuituba* site it was 0.300 ± 0.300 (we were only able to incorporate data from 2 individuals at the *I. tenuituba* site).

Scent Manipulation Data

For the 2012 scent manipulation data, no ANOVA from data over a single week found significant differences in oviposition (eggs per flower censused) among treatments. However, using the combined data across all 4 weeks we found a trend of differences in eggs per flower among all treatments ($F_{7,125}=2.04$, $p=0.0554$). Our four single enantiomer treatments [(+)- α , (-)- α , (+)- β and (-)- β] had a mean eggs per flower that was significantly higher than our control ($F_{1,125}=6.05$, $p=0.0153$) (Figure 1).

Additionally, our (+)- β enantiomer tended to have higher oviposition than the (-)- β enantiomer treatment (Figure 1). However, we also found our control to be significantly lower than our background oviposition rate ($F_{1,125}=6.45$, $p=0.0123$; Figure 1).

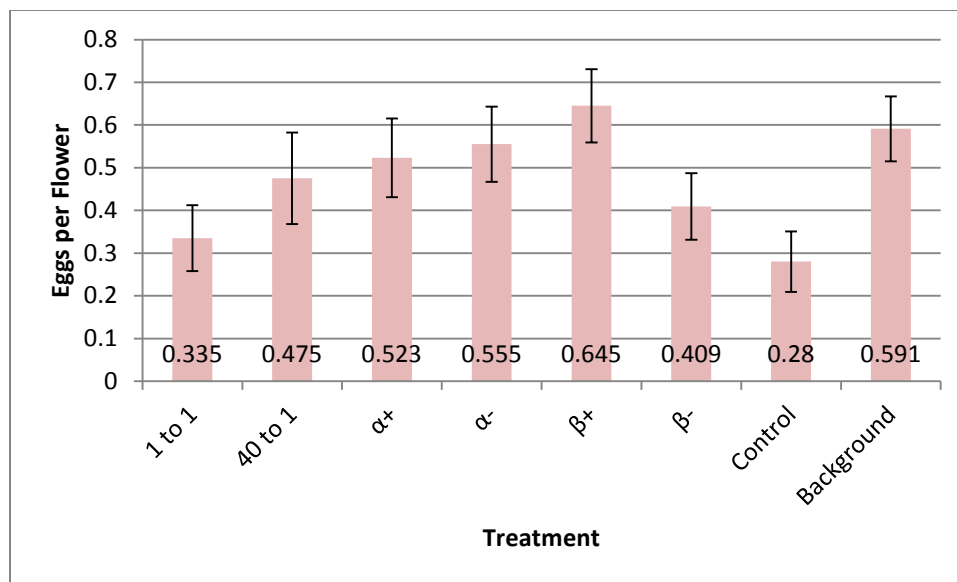


Figure 1. Mean (± 1 standard error) for oviposition by *Delia* (total eggs per flower) for each treatment for 2012 data.

We combined our scent manipulation data from this year with data from the last two weeks of last year's experiment. Although the methods of scent application were different between the two years and the response variable in 2011 was eggs per bud rather than eggs per flower, we found no year x treatment interaction ($F_{4,146} = 1.18$, $p = 0.3212$). The lack of a detectable interaction suggests that there were no differences in patterns of oviposition between the two years. There was evidence of differences in oviposition among treatments for the combined data ($F_{4,146}=3.27$, $p=0.0133$; Figure 2). For the combined two-year data, the (+)- β and

(-)- β enantiomer treatments were significantly different ($F_{1,146}=6.67$, $p=0.0108$), and the single enantiomer treatments were significantly different from the control ($F_{1,146}=5.46$, $p=0.0209$) (Figure 2).

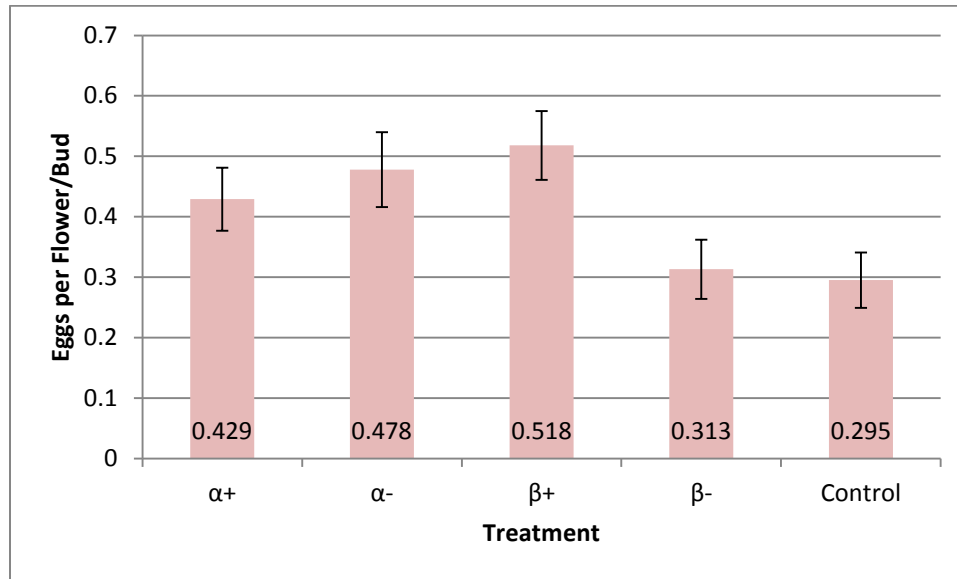


Figure 2. Mean (± 1 standard error) for oviposition by *Delia* (eggs per flower or bud censused) and standard error by treatment for combined 2011-2012 data.

Discussion

We were unable to find statistical evidence that *Delia* oviposition is correlated with any of the morphological floral traits incorporated in our analyses; however, this data set had a relatively small sample size of 35 plants or less depending on the trait. Results from other studies suggest that *Delia* may preferentially oviposit on *Ipomopsis* flowers with wider corollas, indicating that seed predation by *Delia* has the ability to impact selection on floral traits (Campbell *et al.* 2002, Zimmerman and Brody 1998). It is important to note that wider corollas are typical of *I. aggregata* flowers and therefore other factors associated with *I. aggregata* (i.e. additional morphological characteristics or volatile emissions) might also be contributing to this perceived preference of *Delia* for flowers with wider corollas. Although previous studies have

also suggested that traits such as increased sepal width also correlate to increased *Delia* oviposition (Campbell *et al.* 2002), we found no evidence of this for our data, either. Because of the limited number of individuals producing flowers at Poverty Gulch this summer, however, our sample size was restricted. Additionally, if *Ipomopsis* flowers at Poverty Gulch were limited this year because of dry early summer conditions, it is possible that *Delia* were forced to be less selective in terms of floral traits than they might be under more “typical” conditions. For instance, Hainsworth *et al.* (1984) noted that *Delia* typically only lay 1 egg per flower, and if more than 1 egg is laid, usually only one survived. We often noticed flowers containing 2 or more eggs this summer at both of our sites—which may suggest that *Delia* were responding to a potentially limited resource by being less selective in oviposition choices. Indeed, Wallin and Raffa (2004) found that spruce beetles (*Dendroctonus rufipennis*) exhibit density-dependent host selection behaviors, becoming less selective to host characteristics as intraspecific competition pressures increase.

Zimmerman and Brody (1998) found that unexposed *Delia* eggs (eggs completely hidden beneath the sepal) are significantly more likely to develop to larval stage than exposed eggs, which may explain the potential *Delia* preference for flowers with wider corollas and wider sepals. If wider flowers are indeed more susceptible to *Delia* oviposition, conflicting selection pressures from seed predators and pollinators could be in operation on the *Ipomopsis* individuals in this hybrid zone, especially in years where hawkmoths are limited (Campbell 2003). Hummingbirds are more effective pollinators of flowers with wider corollas (Campbell *et al.* 1996)—and at some point there is likely an adaptive morphological balance where increased seed set due to pollination success is countered by loss of seeds due to higher rates of *Delia* oviposition.

Our scent manipulation results from 2012 appear to indicate that our control actually reduced oviposition relative to background rates, and that the addition of the single pinene enantiomer treatments brought oviposition rates back up into the range of background oviposition rates. We are unsure as to why our control of scent-free mineral oil seems to have deterred *Delia*; however, we have scent samples from background, control, and all treatments, and when that data is analyzed using GCMS we may be able to determine if there was contamination, or if a particular component of the mineral oil control may have acted as a potential repellent to *Delia*. Data from the scent manipulation experiment conducted last year did not find any significant differences among treatments when data from that year were analyzed singly, but neither did they find that their control (purified paraffin oil) lowered oviposition. The previous control did have the lowest mean of all treatments over the two weeks analyzed from 2011, although this difference was not significant. Our mineral oil (used as control and as the base for our treatment dilutions) was not labeled as purified, but was labeled odor- and taste-free. A few studies (Poerwanto *et al.* 2011, Nguyen *et al.* 2007, Maganga 1989) have suggested that mineral oil components may act as olfactory deterrents to some insects, including flies. It is also possible that the wooden skewers used to support the emitters, or another part of the emitter apparatus itself discouraged *Delia* oviposition (emitters and stakes were present for control and treatment plants but not for background plants).

However, when combined data from 2011 and 2012 were analyzed, we were able to show that single enantiomer treatments of α - and β -pinene significantly increased oviposition relative to the controls, and the (+)- β pinene treatment was significantly more attractive to *Delia* than the (-)- β pinene treatment. Therefore, combined data from 2011-2012 indicate that *Delia* oviposition behavior does respond to treatments of α - and β -pinene compounds. At the same time, it is also

important to note that even if pinene emissions by *Ipomopsis* prove to influence *Delia* responses, other floral traits and spatial cues may also be important factors in determining oviposition behavior.

To determine whether responses to pinene enantiomers influence selection in the hybrid zone, we first need to know whether the two species differ in enantiomeric composition of pinenes. Scent sampling to date has not shown strong differences between the two *Ipomopsis* species in daytime emission rates of α - and β -pinene (when enantiomers were not separated), but natural hybrids emit less α -pinene than the parental species (Bischoff, Juergens and Campbell, unpub. data). If, as seen in our study, single pinene enantiomers encourage *Delia* oviposition, then decreased α -pinene emissions could make hybrids less susceptible to seed predation. If their sepals and corollas are generally intermediate in width between those of the parental species, they also might experience less oviposition than *I. aggregata*. This lowered oviposition could result in selection for hybrids, in terms of seed predation. However, as hybrids, because of their intermediate floral characteristics, might already be less attractive to hummingbirds than *I. aggregata* and less attractive to hawkmoths than *I. tenuituba*, the result could be selection by pollinators against hybrids (Campbell 2003). Pollinators and seed predators could be generating selection in opposite directions.

Additionally, if the emission rate of one or several enantiomers correlates to nectar production rate or another floral trait (such as corolla width) that might impact pollinators, pinene emissions could indirectly be linked to pollination success. While it is unlikely that hummingbirds respond to pinene emissions at the same level as *Delia*, insect pollinators (solitary bees, lepidopterans, and other insects) could be impacted by differences in the relative amount of the pinene enantiomers (Campbell, Waser and Price 1996). These insect pollinators are not the

main pollinators of either *Ipomopsis* species or their hybrids in Poverty Gulch, but they account for 5-10% of all pollen transfer and their responses to floral scent components could still impact seed production and plant success, especially in sites (or years) where hummingbird visits are less frequent (Campbell, Waser and Price 1996). Volatile emissions could also impact nectar robbing by bumblebees (*Bombus occidentalis*) (Irwin and Dorsett 2002).

Furthermore, *I. aggregata* (but not its sister species) upregulates emission of α -pinene at night (Bischoff, Juergens and Campbell, unpub. data). While the reason for this is unknown, this upregulation could potentially impact hawkmoth visits. Hawkmoths are generally crepuscular pollinators and tend to avoid *I. aggregata* in favor of *I. tenuituba*, which produces indole at night, unlike *I. aggregata* (Bischoff, Juergens and Campbell, unpub. data). Raguso *et al.* (2003) found indole to be a potential signal compound for hawkmoth-pollinated plants. Hummingbirds, on the other hand, typically do not respond to floral scent (Raguso *et al.* 2003). Therefore, differences in floral scent emissions among the two *Ipomopsis* species and hybrids could potentially be reinforcing species boundaries by maintaining and enforcing pollinator specialization.

While results from our 2012 scent manipulation experiment are somewhat difficult to interpret because of the unexpected impact of our control treatment on oviposition, our results, in coordination with the results of similar studies, suggest that *Delia* do indeed exhibit preferences for certain floral traits (whether morphological or chemical) in *Ipomopsis* individuals. This study underscores the necessity of incorporating herbivore species which interact with flowers, as well as pollinators, into investigations of factors influencing the evolution of floral traits. In certain instances, selection pressures from seed predators or other herbivorous insects may even oppose

pollinator-mediated selection pressures, leading to adaptive trade-offs in terms of specific floral traits or suites of traits.

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