

Received Date : 02-Dec-2015  
Revised Date : 20-Jun-2016  
Accepted Date : 22-Jun-2016  
Article type : Original Article

**Running Head:**  $Q_{ST}$ - $F_{ST}$  comparisons for floral traits

Adaptive differentiation in floral traits in the presence of high gene flow in scarlet gilia  
(*Ipomopsis aggregata*)

Elizabeth R. Milano<sup>1</sup>, Amanda M. Kenney<sup>2</sup>, Thomas E. Juenger<sup>1</sup>

<sup>1</sup>The University of Texas at Austin, Department of Integrative Biology, 1 University Station  
C0930, Austin, Texas 78712

<sup>2</sup>St. Edward's University, Department of Biological Sciences, 3001 S. Congress Ave., Austin,  
Texas 78704

Author for correspondence:

*Elizabeth R. Milano*

*Tel: +1 805 795 7368*

*Email: ermilano@gmail.com*

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:

10.1111/mec.13752

This article is protected by copyright. All rights reserved.

**Key Words:** adaptive divergence, floral traits, pollinator visitation,  $Q_{ST}$ , quantitative genetics, *Ipomopsis aggregata*

### **Abstract**

Plant-pollinator interactions are thought to be major drivers of floral trait diversity. However, the relative importance of divergent pollinator-mediated selection versus neutral processes in floral character evolution has rarely been explored. We tested for adaptive floral trait evolution by comparing differentiation at neutral genetic loci to differentiation at quantitative floral traits in a putative *Ipomopsis aggregata* hybrid zone. Typical *I. aggregata* subsp. *candida* displays slender white tubular flowers that are typical of flowers pollinated by hawkmoths and subsp. *collina* displays robust red tubular flowers typical of flowers pollinated by hummingbirds; yet hybrid flower morphs are abundant across the East Slope of the Colorado Rockies. We estimated genetic differentiation ( $F_{ST}$ ) for nuclear and chloroplast microsatellite loci and used a half-sib design to calculate quantitative trait divergence ( $Q_{ST}$ ) from collection sites across the morphological hybrid zone. We found little evidence for population structure and estimated mean  $F_{ST}$  to be 0.032.  $Q_{ST}$  values for several floral traits including corolla tube length and width, color, and nectar volume were large and significantly greater than mean  $F_{ST}$ . We performed multivariate comparisons of neutral loci to genetic correlations within and between populations and found a strong signal for divergent selection, suggesting that specific combinations of floral display and reward traits may be the targets of selection. Our results show little support for historical subspecies categories, yet floral traits are more diverged than expected due to drift

alone. Non-neutral divergence for multivariate quantitative traits suggests that selection by pollinators is maintaining a correlation between display and reward traits.

## **Introduction**

Flowers are extremely diverse and vary in color, scent, size, shape, and pollination reward. These traits are thought to have evolved principally as adaptations for attracting and exploiting pollinators, which in turn evolve characteristics improving their ability to forage on particular flowers. An important aspect of this co-evolutionary process is that it may lead to phenotypic and genetic divergence among plant populations adapted to different pollinator communities and eventually speciation (Grant 1949, 1981; Grant & Grant 1965; Stebbins 1970; Waser *et al.* 1996; Coyne & Orr 2004; Fenster *et al.* 2004). The predominant view is that adaptation to the most abundant or efficient pollinator in an isolated population results in floral divergence, and that subsequent pollinator preference or mechanical isolation will limit intercrossing if diverged populations come into secondary contact (Grant 1949, 1981, 1992a; b; Waser *et al.* 1996; Fenster *et al.* 2004). Co-evolutionary change coupled with the development of pollinator isolation and divergence is arguably one of the most important processes generating diversity in flowering plants (Grant 1994).

The vast majority of plant adaptation studies focus on single traits and univariate analyses. However, organisms are composed of many interrelated phenotypes that are often integrated and function jointly to impact individual fitness (Kirkpatrick 2009; Walsh & Blows 2009). Pollinator effectiveness is likely associated with appropriate matching between floral

traits (e.g., corolla tube length, stigma and anther positioning) and the specific morphological and behavioral characteristics of visiting pollinators (Grant & Grant 1965; Faegri & Pijl 1979; Fenster *et al.* 2004). Likewise, features associated with pollination syndromes suggest strong associations between display and reward characters. A classic example is the commonly observed associations between display characteristics, such as flower color, and nectar rewards for either bird or moth pollination syndromes. The hummingbird pollination syndrome is characterized by short and robust red corolla tubes, little fragrance, and copious production of dilute nectar. In contrast, the hawkmoth pollination syndrome is characterized by a long and slender pale corolla tube, strong fragrance, and low nectar production. These patterns suggest that pollinators may impose strong correlational selection (Lande & Arnold 1983; Sinervo & Svensson 2002; Campbell 2009) on functional floral traits, possibly impacting the evolution of floral trait genetic covariances through either the accumulation of pleiotropic effects or the build-up of linkage disequilibrium. Despite these interesting features, few studies of pollinator-mediated selection and floral adaptation have embraced more sophisticated multivariate analyses (Campbell 2009).

A longstanding goal in plant biology has therefore been to elucidate the factors that constrain or facilitate floral adaptation and that drive pollinator-mediated phenotypic divergence. A number of evolutionary mechanisms can influence phenotypic divergence, and a thorough understanding requires information on the relative importance and consequences of such forces on appropriate spatial and temporal scales (Wade & Goodnight 1998). Natural selection may result in either inter-deme divergence or convergence depending on the form of selection. Although these

evolutionary forces can vary in time and space, many field studies of pollination biology have been conducted within a single sampling location and therefore may miss the complexity or dynamics imposed by population structure and multiple evolutionary forces (Thompson 1994, 1999; Waser *et al.* 1996; Hoeksema & Forde 2008). Consequently, studies integrating across spatial scales and evolutionary processes hold promise for providing a richer understanding of phenotypic change driven by plant-pollinator interactions (Thompson 1994; Wade & Goodnight 1998; Nuismer *et al.* 1999; Sahli *et al.* 2008; Jiménez-Lobato & Núñez-Farfán 2012). Notably, Hopkins *et al.* (2011) and Streisfield and Kohn (2005) compared flower color divergence to divergence in neutral loci to identify a pattern of selection on flower color.

Comparison of the divergence of quantitative traits and neutral genetic markers provides one method for integrating across spatial and temporal scales and for testing the relative importance of neutral versus adaptive processes. This approach, known as  $Q_{ST}$ - $F_{ST}$  comparison, is based on evaluating the degree of among to within population genetic differentiation at both putatively neutral genetic markers and hypothesized adaptive quantitative traits. Differentiation at molecular markers is commonly quantified using the metric  $F_{ST}$ , which at neutral loci is largely influenced by effective population size and the degree of among population migration over evolutionary timescales (Wright 1951).  $Q_{ST}$  is an analogous metric of the genetic differentiation among populations for a quantitative trait that has an additive genetic basis.  $Q_{ST}$  for a neutrally evolving quantitative trait is expected to be, on average, equal to  $F_{ST}$  for a neutral genetic locus (Spitze 1993; McKay & Latta 2002; Whitlock 2008). As such, accurate measures of  $F_{ST}$  at neutral markers can provide a useful null hypothesis that quantitative divergence is explained by

neutral processes alone. For example, if empirical measures of  $F_{ST} \approx Q_{ST}$  we can infer that the observed quantitative trait differentiation could be explained by genetic drift-gene flow balance alone. If  $Q_{ST}$  is significantly less than  $F_{ST}$ , indicating less divergence among populations than expected by genetic drift-gene flow balance, stabilizing selection may be acting to maintain the phenotype across populations. If  $Q_{ST}$  is significantly greater than  $F_{ST}$ , indicating that the focal trait has diverged among populations more than expected by genetic drift-gene flow balance alone, divergent selection may underlie the observed phenotypic divergence among populations. This approach was pioneered by Spitze (1993) and despite the difficulty in accurately measuring  $F_{ST}$  and  $Q_{ST}$  (O'hara & Merilä 2005; Whitlock 2008) the past decade has seen an exponential increase in refinement of the method and application to empirical studies (Whitlock & Guillaume 2009; reviewed in: Le Corre & Kremer 2012; Gilbert & Whitlock 2014). A logical extension of this method incorporates the covariance structure of individual components of a complex trait (Chapuis *et al.* 2008; Martin *et al.* 2008). Moreover, integrating the genetic architecture of complex traits with population structure can help shed light on the evolutionary forces that shape phenotypic divergence.

In this study, we are interested in elucidating the importance of pollinator-mediated selection on the multivariate floral phenotype in scarlet gilia (*Ipomopsis aggregata* Polemoniaceae). Scarlet gilia is an ecologically well-studied wildflower with a wide geographical range across most of the western United States. The *I. aggregata* species complex is composed of at least eight subspecies, several occurring sympatrically, with clear contact zones and regions of hybridization (Grant 1956; Wolf & Soltis 1992; Porter & Johnson 2000). Co-evolutionary

Accepted Article  
interaction with pollinators (hummingbirds, hawkmoths, bees, butterflies) is likely an important driver of diversification and hybridization within the *I. aggregata* complex (Grant 1992b). Field studies of *I. aggregata* subsp. *aggregata* arguably provide the best evidence for the occurrence and strength of pollinator-mediated selection on floral morphology in natural populations. For example, long-term studies at the Rocky Mountain Biological Laboratory (RMBL) in western Colorado have documented that pollen-limitation coupled with variation in pollinator visitation and efficiency can impose strong natural selection on floral characters including flower size and shape (Campbell 1989, 1991, 1996), inflorescence display (Campbell 1991; Mitchell 1994), nectar production (Mitchell & Waser 1992; Mitchell 1993), and timing of male and female reproduction (Campbell 1991, 1997; Campbell *et al.* 1996). Studies of an *Ipomopsis* hybrid zone at RMBL (*I. aggregata* ssp. *aggregata* x *I. tenuituba*) have also detailed variable selection imposed by hummingbird and hawkmoth pollinators on floral morphology (Campbell *et al.* 1997, 1998; Melendez-Ackerman 1997; Melendez-Ackerman & Campbell 1998; Campbell 2004). Surprisingly little work on plant-pollinator interaction has been pursued in other *I. aggregata* populations, subspecies, or hybrid zones.

We extend earlier studies by exploring patterns of molecular marker and quantitative trait differentiation occurring on the East Slope of the Rocky Mountains. In particular, we evaluate the population structure and floral trait differentiation in local occurrences of *I. aggregata* subsp. *candida* (Rydb.) V.E. Grant & A.D. Grant and *I. aggregata* subsp. *collina* (Greene) Wilken & Allard (hereafter referred to as *candida* and *collina*) using univariate ( $Q_{ST}$ - $F_{ST}$ ) and multivariate (genetic covariance matrix deconstruction) comparisons to test for departure from expectations

of neutral evolution. The flowers of subspecies *candida* exhibit classic hawkmoth pollination syndrome characteristics and the flowers of subspecies *collina* exhibit classic hummingbird pollination syndrome characteristics (Figure 1 a & b). *Collina* flowers typically produce a large amount of dilute nectar in comparison to *candida* flowers, again matching the typical hummingbird and hawkmoth associated suites of traits (Fenster *et al.* 2004; Mitchell 2004). The genetic and evolutionary history of these morphologically recognized subspecies is unknown.

*I. a. candida* and *collina* hybridize across much of the East Slope of the Rockies (Figure 2). This hybrid zone was first investigated with respect to the introgression of floral characters by Wilken and Allard (1986), and Grant and Wilken (1987) and Elam and Linhart (1988). One hypothesis is that the *candida* morph diverged from a *collina*-like ancestor during the last glacial maximum as an isolated allopatric population at low elevation on the shortgrass prairie. Following glacial retreat and climate change, *candida* populations are thought to have extended their range from the prairie to higher elevations and thus come into secondary contact with *collina* (Grant & Wilken 1987), generating a complex mosaic of hybridization and introgression throughout the East Slope. Given this hypothesis, we would predict both neutral and quantitative trait divergence, depending on the degree and extent of isolation and reproductive incompatibilities. Alternatively, *candida* and *collina* may be undergoing primary divergence ostensibly by pollinator-mediated disruptive selection on floral types (Wolf *et al.* 1991). This scenario may result in substantially stronger quantitative trait divergence with potentially little or no neutral marker divergence because of ongoing gene flow.



In this study we used an integrative approach to address hypotheses of differentiation across the broad geographical distribution of *I. aggregata* on the Front Range of the Rockies. First, we utilized putatively neutral microsatellite markers to characterize molecular genetic differentiation, population structure, and isolation-by-distance across occurrences in the Front Range. Second, we used a greenhouse common garden study to estimate quantitative genetic structure and evaluate population differentiation of floral and life history traits. Together, these data allowed inference on the potential adaptive differentiation of floral characters across a broad geographical region and shed light on earlier hypotheses concerning the *candida/collina* hybrid zone.

## Materials and Methods

### *Study System and Sampling Locations*

Scarlet gilia is a common monocarpic, perennial wildflower of western montane habitats. It is frequently found in large grassy meadows and along roadsides interspersed with small patches of ponderosa pine forest occurring from 1740 m to over 3050 m elevation on the East Slope of the Rocky Mountains in Colorado (Wilken & Allard 1986). After germination, scarlet gilia grows as a hardy rosette for 2–5 years until it bolts an indeterminate flowering stalk, reproduces, and dies. Scarlet gilia has showy inflorescences with flowers that are hermaphroditic, protandrous, and self-incompatible (Juenger 1999). The flowers of subspecies *candida* and *collina* and their hybrids range in color from pure creamy white, light shades of pink, to vivid scarlet red (Figures 1a, 1b, 2). They are pollinated by the white-lined hawkmoth (Sphingidae: *Hyles lineata*) and, to a lesser extent, by resident and migratory hummingbirds, including Broad-tailed Hummingbirds

(*Selaphorus platycerus*), Rufous Hummingbirds (*Selaphorus rufus*), and Calliope Hummingbirds (*Stellula calliope*) (Elam & Linhart 1988) (T. Juenger, A. Kenney & E. Milano, personal observation).

We studied 27 occurrences of scarlet gilia from locations in Colorado (Table S1, Figure S1). The occurrences were identified from prior studies (Wilken & Allard 1986; Grant & Wilken 1987) along with new locations that we identified from extensive field work on the Front Range. Sampled locations covered low elevation foothills, montane grasslands, and mixed forest habitats in the Northern and Southern Front Range, Sangre De Cristo Range, and Wet Mountains. The locations were selected to be representative of the range trait distributions of the focal taxa.

#### *Neutral Genetic Markers*

At 22 locations (Figure 3, Table S1), leaf tissue was collected from 8-12 randomly selected plants. Genomic DNA was extracted from leaf tissue using a DNeasy Plant tissue kit (Qiagen). Genomic DNA was genotyped at 11 nuclear microsatellite loci derived from 4 custom enrichment microsatellite libraries (Stearns *et al.* 2008). This set of microsatellites covers 6 of 7 *I. aggregata* chromosomes, as determined by standard linkage analysis (Kenney 2011). In addition, each sample (from 19 of the original 22 locations) was genotyped at 5 chloroplast microsatellites (Provan *et al.* 2001). Polymerase chain reaction (PCR) amplification was carried out in a Peltier DNA Engine Tetrad 2 thermal cycler (BioRad) in 5uL of reaction mixture containing 20 ng of template DNA, ddH<sub>2</sub>O, 0.8 μM dNTPs, 1 × NH<sub>4</sub> reaction buffer (Bioline),

1.5  $\mu\text{M}$   $\text{MgCl}_2$ , 0.01  $\mu\text{M}$  forward primer with a 19 bp M13F tail (CACGACGTTGTAAAACGAC), 0.15  $\mu\text{M}$  reverse primer, 0.15  $\mu\text{M}$  M13- HEX, FAM or Tamara, and 0.01 U of *Taq* DNA Polymerase (Bioline). DNA was initially denatured at 94.0 °C for 2 min, cycled 35 times at 94.0 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and then finished with a final extension step at 72 °C for 10 min. Amplification products were detected on an Applied Biosystems 3100 DNA Sequencer (Life Technologies), and fragments called and analyzed using GeneMarker (v. 1.3) software.

#### *Quantitative Phenotypic Traits*

A dam half-sib design was implemented for our quantitative genetic studies by field collecting open-pollinated fruits from 182 maternal plants from 16 locations (Figure S1, Table S1). These seeds were cold stratified for 6 weeks in shallow trays of sand in a cold room (4 °C) at the University of Texas at Austin. Seeds were then placed in the UT Austin greenhouse for germination. Once true leaves were present, seedlings were transplanted into cone-tainers (Steuwe & Sons, SC10, 164ml volume) with a 3:3:4 ratio of sand, turface (Profile Products LLP), and Pro-mix (Premier Tech) as a potting medium and randomized across growing trays and benches. Plants were grown under long day conditions (16h day/8h night) using supplemental overhead high pressure sodium lighting and standard greenhouse conditions based on evaporative cooling. After 4 months of vegetative rosette growth, plants were returned to a 4°C cold room for 4 months of vernalization to simulate a natural overwintering. Following cold vernalization, plants were subsequently transferred to the greenhouse for their final growing season. Plants bolted a growing inflorescence and flowered over a 158-day period (average 67.7

+/- 0.96 SE days) following reintroduction to the greenhouse. In total, 745 plants were established for our greenhouse studies forming 180 maternal sibships (mean family size: 4.1 +/- 3.78 SD progeny) across 16 collection locations (Table S1).

Siblings were phenotyped for a series of traits associated with floral display, reward, and life history. Display traits and reward traits were chosen to best characterize the variation in *candida/collina* flowers. Display traits measurements included corolla tube length, corolla tube width at the widest point, length of longest petal lobe, and flower color, on three flowers per plant. The color variation observed in *Ipomopsis* flowers is primarily the product of anthocyanin pigments (Harborne & Smith 1978). Pigments from harvested corolla tubes were extracted with 200 proof methanol and quantified on a standard plate reader (Beckman DTX880) at a wavelength of 492 nm, the major absorbance wavelength of the primary anthocyanin pigment, pelargonidin. Dry weight of the floral tissue was used to standardize the absorbance intensity as a quantitative estimate of color. Reward trait measurements included nectar volume and concentration on three flowers per plant. To standardize measurements and avoid evaporation, elongating buds were taped shut in the afternoon the day before they were to open. Forty-eight hours later, each flower was carefully removed and nectar was extracted from the base of the corolla tube with a capillary tube (Drummon Scientific). Nectar concentration was quantified using a hand-held refractometer (Eclipse 45-03, Bellingham and Stanley Ltd.). All experimental plants were well-watered to reduce the potential impact of water limitation on nectar production.

Data were also collected for two traits associated with life history. Flowering time was measured as the number of days of growth in the greenhouse following vernalization, to the date of the first open flower. *Ipomopsis* grows as a rosette that develops a hardy rootstock before flowering. Rootstock diameter is a strong predictor of overall plant size and is correlated with plant height and flower production (Juenger & Bergelson 1998). Rootstock diameter was measured at the end of the experiment after 7 months of growth in the greenhouse.

#### *Population Genetic Data Analysis*

Descriptive statistics for microsatellites were generated using the R package *adegenet* (Jombart 2008, version 1.3-4) based on allele designations from fragment size determinations. Here we provide a brief summary of the nuclear microsatellite data used for population genetic analysis.

Across the entire sample of individuals (n=230), the mean number of alleles at nuclear microsatellites was 46 (+/- 6.72 SE); the mean observed heterozygosity was 0.633 (+/- 0.042 SE); and the mean inbreeding coefficient ( $F_{IS}$ ) was 0.257 (+/- 0.034 SE). After Bonferroni correction only two collection locations had one locus each that exhibited a departure from Hardy-Weinberg expectations. Population structure was assessed by principal component analysis (PCA) and discriminant function analysis (DAPC) in *adegenet* (Jombart & Devillard 2010) and with the Bayesian clustering program STRUCTURE (Pritchard *et al.* 2000). STRUCTURE analyses were completed with a model specifying admixture, no location prior, a 10,000 iteration burn-in, and a 20,000 iteration MCMC. Results were evaluated with Structure Harvester and optimal K determined using the delta method (Evanno *et al.* 2005; Earl & VonHoldt 2012). Isolation-by-distance was evaluated using a Mantel test to assess the

Accepted Article  
correlation between a matrix of Nei's pairwise  $F_{ST}$  and Euclidian geographic distance. Haplotype analysis for chloroplast microsatellites was performed in the R package *haplotypes v1.0* using a 99% parsimonious cutoff for the network analysis.

### *Quantitative Genetic Analysis*

The quantitative phenotypic traits were box-cox transformed to meet the requirement of normality for all analyses. Traits were also assumed to have purely additive modes of inheritance and we did not make any assumptions about historical sub-species divisions.  $Q_{ST}$ - $F_{ST}$  comparisons must be completed using a rigorous statistical test (Merilä & Crnokrak 2001; O'hara & Merilä 2005); in particular, a key assumption is that the distribution of  $Q_{ST}$  for neutral traits is equivalent to that of neutral  $F_{ST}$  markers (Whitlock 1999, 2008; Leinonen *et al.* 2013). Whitlock and Guillaume (2009) developed a method using parametric simulations and bootstrapping to predict the null distribution of neutral  $Q_{ST}$  and compare the observed  $Q_{ST}$  to the tails of the simulated null distribution. An extension of this procedure allows for an unbalanced open-pollinator design to calculate observed  $Q_{ST}$  (Gilbert & Whitlock 2014). This method allows for a maternal pedigree of half-sib design with no need to know sire, which is a common design for outcrossing plant populations. We centered our analyses on a half-sib design based on a study that found multiple paternity in over two-thirds of *Ipomopsis aggregata* fruits, with number of sires averaging 4 per fruit (Campbell 1998). Calculation of  $F_{ST}$ ,  $Q_{ST}$ , and associated distributions and confidence intervals were completed using the R package *QstFstComp* provided by Gilbert and Whitlock (2014).

### *Multivariate analysis*

We first performed a PCA on phenotypic traits to visualize differences between collection sites.

We then performed two multivariate methods to test for selection. The first is a natural extension of the  $Q_{ST}$ - $F_{ST}$  comparison which used genetic covariance matrices within and between populations to test for non-proportionality (Martin *et al.* 2008). The second used existing neutral markers and quantitative traits to simultaneously estimate ancestral population parameters and derive theoretical null expectations for each population against which we tested for signatures of divergent or stabilizing selection in observed populations (Ovaskainen *et al.* 2011).

We performed a multivariate extension of the  $Q_{ST}$ - $F_{ST}$  analysis by comparing the among population genetic covariance matrix ( $\mathbf{G}_B$ ) to the average within population genetic covariance matrix ( $\mathbf{G}_W$ ) using the two-part test developed by Martin *et al.* (2008). For the multivariate analyses we use the term ‘population’ to refer to the collection sites. We used a MANOVA to estimate  $\mathbf{G}_W$  and  $\mathbf{G}_B$  from genetic family means. Under neutral expectations  $\mathbf{G}_W$  and  $\mathbf{G}_B$  are expected to be proportional where the proportionality coefficient,  $\rho_{st} = 2 * F_{ST} / (1 - F_{ST})$  (Rogers & Harpending 1983; Martin *et al.* 2008). In this two-part test we calculated a proportionality coefficient ( $\rho_{st}$ ) such that  $\mathbf{G}_B = \rho_{st} * \mathbf{G}_W$ . We first tested if  $\rho_{st}$  for quantitative traits ( $G\rho_{st}$ ) was significantly greater than  $\rho_{st}$  calculated from the neutral marker loci ( $N\rho_{st}$ ). If  $G\rho_{st} > N\rho_{st}$  then we inferred deviation from neutral expectations. We then tested  $\rho_{st}$  itself using a likelihood ratio test where  $H_0: \mathbf{G}_B = \rho_{st} * \mathbf{G}_W$ , and  $H_1: \mathbf{G}_B$  and  $\mathbf{G}_W$  are completely unrelated. We used a Bartlett correction of the likelihood ratio to account for small degrees of freedom. This second part of the test gives insight into the relative structure and orientation of  $\mathbf{G}_B$  and  $\mathbf{G}_W$ . Calculations were

performed using customized R scripts from Martin et al. (2008) and we applied a matrix bending correction to  $\mathbf{G}_B$  to make it positive-definite. Finally, we performed eigen deconstruction to compare trait loadings on the major principal components of  $\mathbf{G}_B$  and  $\mathbf{G}_W$ .

The second multivariate test to detect signatures of divergent or stabilizing selection in quantitative traits is similar to the animal model (Kruuk 2004) in that we assumed that the additive variance among relatives, as measured by the animal model, holds at the population level (Ovaskainen *et al.* 2011). The genetic value of an individual now becomes the genetic mean of a population and the coancestry coefficient between individuals is now the coancestry coefficient between individuals belonging to different populations. This test, developed by Ovaskainen et al. (2011), is based on the assumption that all populations are derived from a common ancestral population. We use Bayesian inference to estimate a genetic coancestry matrix based on neutral markers and a genetic covariance matrix based on quantitative traits. We then use these ancestral matrices to derive expectations of evolution under a null model of random genetic drift for *each* population. This is in contrast to the previous test where we averaged  $F_{ST}$  and  $\mathbf{G}_W$  across populations. We then explicitly test for neutrality using the likelihood of the observed pattern given the theoretical expectation based on random genetic drift. The signature of selection test statistic,  $S$ , is defined as the probability that the actual pattern of additive genetic divergence is less than that of the randomized realizations of additive genetic divergence. A value of  $S \approx 1$  means that the observed pattern is unlikely under neutrality. The typical pattern under neutrality is  $S \approx 0.05$ . In the case of stabilizing selection, the value of actualized divergence is lower than expected due to random drift so  $S \approx 0$ . To perform this test we



used a subset of 11 populations that had both neutral marker and quantitative trait information. (Table S1, Figure S1). The analyses were completed using the R packages *RAFM* to calculate the posterior distributions of drift and gene flow from each population, and *driftsel* to calculate the expected differences in population trait means and perform the neutrality test using 10,000 random draws (Karhunen *et al.* 2013).

## Results

### *Population genetic structure*

Neutral marker analyses revealed little genetic differentiation or structure across the sampled region. Overall, nuclear  $F_{ST}$  was low (mean  $F_{ST} = 0.032$ , 95% CI: 0.023-0.042), but increased with geographic distance, indicating isolation-by-distance (IBD) primarily along a north-south axis (Figure S2). A Mantel test for IBD revealed a significant correlation between genetic and geographic distance matrices for nuclear markers ( $R^2 = 0.623$ ;  $p < 0.001$ ; 9,999 permutations) and a weaker yet significant pattern for chloroplast markers ( $R^2 = 0.290$ ;  $p = 0.0065$ ; 9,999 permutations). We identified 27 unique chloroplast haplogroups, 19 (70.4%) of which were unique to a single population. The two most common haplogroups represent 40% of the individuals sampled. These two haplogroups are only differentiated by a single chloroplast locus and are present in 12 out of 19 total populations and show no clear association with floral morphological differentiation (Figure S3, Table S2).

Results from a DAPC of nuclear markers found  $K=2$  clusters that do not correspond to putative sub-species divisions, instead suggesting a geographic trend from north to south (Figure 3, Table S3). A STRUCTURE analysis did not find a significant number of  $K>1$  clusters. A PCA of the molecular data showed little differentiation between the populations (Figure 4a), whereas a PCA of floral traits revealed distinct clusters of populations (Figure 4b, Table S4). These results suggest a high degree of nuclear gene flow between locations and are consistent with relatively long range pollen dispersal by animal pollinators, large effective population sizes, and little reproductive isolation among floral syndromes.

#### *Q<sub>ST</sub> and F<sub>ST</sub> comparison*

We found  $Q_{ST}$  for several floral traits to be greater than mean  $F_{ST}$  (0.032, 95% CI: 0.023-0.042), indicating that the traits are significantly more differentiated between populations than expected from drift alone (Figure 5). The floral display and reward traits with significant  $Q_{ST}$  values are corolla tube width, corolla tube length, flower color, and nectar volume. We did not find significant among population genetic variation for petal lobe length or nectar concentration.

Additionally, rootstock diameter and flowering time show  $Q_{ST}$  signals consistent with neutral evolution. The conclusions drawn from our  $Q_{ST}$ - $F_{ST}$  comparisons are robust to analysis assumptions based on sibship relatedness.  $Q_{ST}$  values for corolla tube length, corolla tube width, flower color, and nectar volume were consistently significantly larger than  $F_{ST}$  whether our dam design analysis considered families to be composed of half (relatedness = 0.25) or full (relatedness = 0.50) sibships (data not shown). Our conclusions are also robust to an unbalanced sampling design. Our original  $Q_{ST}$  dataset had an excess of populations without matching neutral

marker information and likewise for the  $F_{ST}$  dataset (Figure S1). We removed the unmatched populations from the analysis (indicated in Table S1) and found the conclusions to be qualitatively consistent with those of the full dataset (Figure S4).

### *Multivariate Tests for Neutrality*

We extended the univariate  $Q_{ST}$ - $F_{ST}$  test into multivariate trait space by incorporating the genetic variance-covariance architecture of the floral traits that is otherwise lost in a univariate analysis.

We estimated the among ( $\mathbf{G}_B$ ) and average-within ( $\mathbf{G}_W$ ) population covariance matrices and found stark differences in the structure of genetic covariation (Table 1). The dominant covariance structure among populations ( $\mathbf{G}_B$ ) was indicative of the expected pollination morphotypes; either red, short, and wide flowers with abundant nectar or white, long, and thin flowers with sparse nectar. In contrast, the major components of quantitative trait variation within populations ( $\mathbf{G}_W$ ) were petal lobe length and nectar concentration. We found  $G_{\rho_{st}}$  for quantitative traits (0.43, 95% CI: 0.33-0.61) was significantly greater than  $N_{\rho_{st}}$  for neutral marker loci (0.066, 95% CI: 0.050-0.084) so we rejected proportionality between  $\mathbf{G}_B$  and  $\mathbf{G}_W$  (Bartlett corrected  $p$ -value $<0.0001$ ). An eigen-analysis of the two genetic matrices illustrated the non-proportionality of the covariance structure (Figure 6). We observed large differences in both the amount of variation described by the first two principal component (PC) axes, and the trait loadings on each. PC 1 and PC 2 described almost 100% of the variation in  $\mathbf{G}_B$  and were comprised of the same 4 significant  $Q_{ST}$  traits. However, PC 1 and PC 2 described only 61.5% of the variation in  $\mathbf{G}_W$ , and were primarily comprised of petal lobe length, nectar concentration and

to a lesser extent, nectar volume. The correlation coefficient ( $r$ ) between  $G_B$  and  $G_W$  PC1 is -0.92 and for PC2 is -0.33.

In our second multivariate approach we estimated a population level coancestry matrix (Figure 7a), based on neutral loci, and an ancestral additive genetic covariance matrix based on neutral expectations of quantitative traits. We then tested each observed population against a null model based on theoretical expectation under random genetic drift and calculated a selection statistic,  $S$  where  $S < 0.05$  indicates stabilizing selection and  $S > 0.95$  indicates directional selection. We found strong evidence for divergent selection across populations ( $S = 1.0$ , Ovaskainen et al. 2011) for a multivariate model that included all 6 floral traits. We visually represent observed trait divergence between populations in relation to the estimated ancestral population (A), and expectation under drift for three pairs of traits (Figure 7). Populations that fall inside their respective 50% confidence ellipses are exhibiting neutral divergence or possible stabilizing selection whereas populations outside of their corresponding ellipses are exhibiting directional selection. We found that the majority of populations exceeded expectations of neutral divergence for trait pairs including corolla tube length, corolla tube width, color, and nectar volume (Figures 7b-c) indicating evidence for multivariate divergence. However, most populations fell inside the 50% neutrality ellipse for petal lobe length and nectar concentration (Figure 7d).

## Discussion

Plant-pollinator coevolution is thought to be a primary driver of plant population divergence and ultimately plant speciation. Here, we explored population genetic structure and quantitative genetic divergence in floral traits in a putative *I. aggregata* hybrid zone. We found little genetic differentiation or population structure along traditional subspecies designations. Overall, nuclear  $F_{ST}$  was low and genetic PCA, discriminant function, and Bayesian clustering approaches found no clear groupings that are strongly coincident with flower color or other floral morphological traits. At best, nuclear microsatellite markers exhibited isolation-by-distance that is primarily associated with a latitudinal axis. We also found no evidence of distinct chloroplast haplotypes that correspond with subspecies designations. Together, these data suggest *I. aggregata* demes in the Front Range of the Rockies experience high levels of gene flow and relatively little reproductive isolation among demes, including between demes with considerably diverged suites of floral traits. These results are consistent with an earlier allozyme study that found high rates of gene flow between *candida* and *collina*, and treated *candida* as a geographic race rather than a subspecies (Wolf & Soltis 1992). These molecular data also suggest that the dominant pollinators, including hummingbirds and hawkmoths, likely have low fidelity to color and do not exhibit strong ethological isolation but are rather flexible in their preferences. This is consistent with earlier findings of Elam and Linhart (1988) who observed hummingbirds and hawkmoths visiting all color morphs along the Front Range, and personal observations from our own fieldwork. As such, we find evidence for a very recent and only shallow divergence of ancestral *candida/collina* populations prior to widespread admixture or alternatively, a model of primary phenotypic divergence and polymorphism in sympatry. Either model suggests a rather rapid

evolution of the hawkmoth syndrome morph from the presumed hummingbird syndrome ancestor.

In contrast to our findings in population structure,  $Q_{ST}$  analyses reveal substantial among-population differentiation in floral traits including corolla tube length and width, color, and nectar volume.  $Q_{ST}$  values estimated from common garden studies were significantly larger than nuclear  $F_{ST}$  values, supporting a model of adaptive among-population divergence. Multivariate analyses further support the notion of adaptive differentiation of floral traits. In particular, comparisons of the average among ( $G_B$ ) and within ( $G_W$ ) population genetic covariance matrices rejected proportionality – an expectation of neutral divergence (Martin *et al.* 2008). Similarly, analyses that used inferred ancestral population states to test for divergence beyond that expected from neutral processes provide strong evidence for selective divergence in multivariate trait space. Collectively, these results suggest that suites of floral traits have been the targets of selection – a hypothesis that would be further supported by the observation of correlational selection on floral traits in the field. Phenotypic manipulation studies in populations of *I. aggregata* on the Western Slope of the Rockies have found evidence for correlational selection on floral traits along expected pollinator trait preference axes (Campbell 2004, 2009; Bischoff *et al.* 2014).

Response to selection is not just controlled by genetic variation but by genetic covariation (Lande & Arnold 1983; Blows & Hoffmann 2005), and defined by the principal components of the genetic covariance matrix (Blows 2007). In this study we found non-proportionality for  $G_B$

Accepted Article

and  $\mathbf{G}_W$ , implying not only that divergence between populations is not occurring at the neutral rate, but also is different across spatial scales. The first two PCs in  $\mathbf{G}_B$  accurately describe the phenotypic divergence we observe in floral traits between *candida* and *collina*. However, the first two PCs for the average  $\mathbf{G}_W$  matrix describe much less of the variation than  $\mathbf{G}_B$  and are comprised primarily of traits that have little genetic variation. This implies response to selection at the within deme spatial scale is different than response to selection across the range. The results from the molecular markers indicate that this is one large admixed population so we should expect the overall *candida/collina* hybrid zone to respond in the dimensions of  $\mathbf{G}_B$ . This discrepancy between genetic covariance matrices suggests population structure and admixture estimates are important considerations in designing experiments that properly sample the diversity of a focal gene pool. If we had only studied a single occurrence or local deme, our interpretation of response to selection in the *candida/collina* hybrid zone might have been substantially different.

We found that the major genetic axis of the among-population genetic covariance matrix corresponded to strong genetic covariances between corolla tube length and width, flower color, and nectar abundance. This indicates strong genetic correlations between the display and reward components of the floral phenotype are maintained in the hybrid zone. Genetic correlations can be the result of pleiotropy, physical genetic linkage, or linkage disequilibrium (LD) resulting from correlated selection pressures (Sinervo & Svensson 2002). Pleiotropy occurs when individual loci have multiple phenotypic effects whereas LD builds up over time due to the non-random association of alleles at different loci. This non-random association can be broken after

sufficient random mating and recombination whereas pleiotropic interactions persist. If the genetic correlations between display and reward traits are due to LD then we can assume that they have built up due to strong and persistent pollinator-mediated correlational selection, indicating that pollinator display is an honest indicator of reward. This may support strong adaptive divergence along the expected hummingbird-hawkmoth specialization axes.

In summary, our analyses of genetic differentiation reveal a somewhat surprising pattern. On the one hand, we observe little molecular genetic differentiation in neutral loci across a fairly large sampling of occurrences spanning elevation and habitat diversity. In contrast, we observed remarkable genetic differentiation in floral reward and display traits, in line with the correlated trait evolution expected in response to divergent pollinator-mediated selection. Given the extensive gene flow inferred, the pollinator-mediated selection must be remarkably strong over evolutionary time scales to withstand the potential swamping and homogenization of allelic differentiation at loci underlying floral traits. Other *Ipomopsis* systems such as those on the Western Slope of the Colorado Rockies do show more reproductive isolation, perhaps due to longer periods of allopatric isolation or more complex reproductive isolation (Campbell 2004). Interestingly, in the *candida/collina* case, the adaptive evolution of pollinator syndromes does not appear to result in the evolution of strong reproductive isolation or generate general barriers to gene flow and suggests an opportunity to identify the diverged floral traits through genome sequencing and outlier  $F_{ST}$  tests (Vitti *et al.* 2013; Lotterhos & Whitlock 2014). Possible mechanisms constraining speciation could include the prevalence of morphologically “hybrid” populations that act as a gene flow bridge through low pollinator fidelity and low ethological



isolation, inconsistent pollinator preferences over ecological and evolutionary timescales, or complex temporal dynamics generated by fluctuating abundances of plants and pollinators from year to year. As such, pollinator-mediated selection alone may be a relatively weak force for ecological speciation in the sympatric occurrences of *Ipomopsis aggregata* on the East Slope of the Rockies.

### Acknowledgements

We thank R. Hopkins, B. Campitelli and 2 anonymous reviewers for comments on early drafts of this manuscript. We thank D. Butler, W. Butler, and S. Boles for help with genomic DNA extractions and microsatellite genotyping of the study samples. A. Nassiri, B. Wong, B. Straub and J. Fuller provided horticultural care and phenotyping expertise on experimental plants. M. Whitlock gave helpful advice on statistical analyses, especially related to the estimation of  $Q_{ST}$ . TEJ was supported by an NSF Career Award (DEB-0546316).

### References

- Bischoff M, Raguso RA, Andreas J, Campbell DR (2014) Context-dependent reproductive isolation mediated by floral scent and color. *Evolution*, **69**, 1–13.
- Blows MW (2007) A tale of two matrices: multivariate approaches in evolutionary biology. *Journal of Evolutionary Biology*, **20**, 1–8.
- Blows MW, Hoffmann AA (2005) A reassessment of genetic limits to evolutionary change. *Ecology*, **86**, 1371–1384.
- Campbell DR (1989) Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. *Evolution*, **43**, 318–334.
- Campbell DR (1991) Effects of Floral Traits on Sequential Components of Fitness in *Ipomopsis aggregata*. *The American Naturalist*, **137**, 713–737.
- Campbell DR (1996) Evolution of Floral Traits in a Hermaphroditic Plant: Field Measurements of Heritabilities and Genetic Correlation. *Evolution*, **50**, 1442–1453.
- Campbell DR (1997) Genetic correlation between biomass allocation to male and female functions in a natural population of *Ipomopsis aggregata*. *Heredity*, **79**, 606–614.
- Campbell DR (1998) Multiple paternity in fruits of *Ipomopsis aggregata* (Polemoniaceae). *American Journal of Botany*, **85**, 1022–1027.

- Campbell DR (2004) Natural selection in *Ipomopsis* hybrid zones: Implications for ecological speciation. *New Phytologist*, **161**, 83–90.
- Campbell DR (2009) Using phenotypic manipulations to study multivariate selection of floral trait associations. *Annals of Botany*, **103**, 1557–1566.
- Campbell DR, Waser NM, Melendez-Ackerman EJ (1997) Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *American Naturalist*, **149**, 295–315.
- Campbell DR, Waser NM, Price M V. (1996) Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. *Ecology*, **77**, 1463–1472.
- Campbell DR, Waser NM, Wolf PG (1998) Pollen Transfer By Natural Hybrids and Parental Species in an *Ipomopsis* Hybrid Zone. *Evolution*, **52**, 1602–1611.
- Chapuis E, Martin G, Goudet J (2008) Effects of selection and drift on G matrix evolution in a heterogeneous environment: A multivariate Qst-Fst test with the freshwater snail *Galba truncatula*. *Genetics*, **180**, 2151–2161.
- Le Corre V, Kremer A (2012) The genetic differentiation at quantitative trait loci under local adaptation. *Molecular Ecology*, **21**, 1548–1566.
- Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland, MA.
- Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.
- Elam D, Linhart Y (1988) Pollination and Seed Production in *Ipomopsis aggregata*: Differences Among and Within Flower Color Morphs. *American Journal of Botany*, **75**, 1262–1274.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, **14**, 2611–2620.
- Faegri K, Pijl L van der (1979) *The principles of pollination ecology*, Volume 1978. Elsevier Science Limited.
- Fenster C, Armbruster W, Wilson P, Dudash M, Thomson J (2004) Pollination Syndromes and Floral Specialization. *Annual Review of Ecology and Systematics*, **35**, 375–403.
- Gilbert KJ, Whitlock MC (2014) QST-FST comparisons with unbalanced half-sib designs. *Molecular Ecology Resources*, **15**, 262–267.
- Grant V (1949) Pollination systems as isolating mechanisms in angiosperms. *Evolution*, **3**, 82–97.
- Grant V (1956) A synopsis of *Ipomopsis*. *Aliso*, **3**, 351–362.
- Grant V (1981) *Plant speciation, 2nd edition*. Columbia University Press, New York, New York.
- Grant V (1992a) Systematics and Phylogeny of the *Ipomopsis aggregata* Group (Polemoniaceae): Traditional and Molecular Approaches. *Systematic Botany*, **17**, 683–691.
- Grant V (1992b) Floral isolation between ornithophilous and sphingophilous species of *Ipomopsis* and *Aquilegia*. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 11828–11831.
- Grant V (1994) Modes and origins of mechanical and ethological isolation in angiosperms. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 3–10.
- Grant V, Grant KA (1965) *Flower pollination in the phlox family*. Columbia University Press, New York.
- Grant V, Wilken D (1987) Secondary Intergradation between *Ipomopsis aggregata candida* and

- I. a. *collina* (Polemoniaceae) in Colorado. *Botanical Gazette*, **148**, 372–378.
- Harborne JB, Smith DM (1978) Correlations between Anthocyanin Chemistry and Pollination Ecology in the Polemoniaceae. *Biochemical Systematics and Ecology*, **6**, 127–130.
- Hoeksema JD, Forde S (2008) A meta-analysis of factors affecting local adaptation between interacting species. *American Naturalist*, **171**, 275–290.
- Hopkins R, Levin DA, Rausher MD (2011) Molecular Signatures of Selection on Reproductive Character Displacement of Flower Color in *Phlox drummondii*. *Evolution*, **66**, 469–485.
- Jiménez-Lobato V, Núñez-Farfán J (2012) Population differentiation in floral characters of *Datura innoxia* Mill. *Evolutionary Ecology Research*, **14**, 1015–1038.
- Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics (Oxford, England)*, **24**, 1403–1405.
- Jombart T, Devillard S (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**.
- Juenger TE (1999) The Ecology and Evolution of Species Interactions in the Scarlet Gilia, *Ipomopsis Aggregata*, System. University of Chicago.
- Juenger TE, Bergelson J (1998) Pairwise versus diffuse natural selection and the multiple herbivores of scarlet gilia, *Ipomopsis aggregata*. *Evolution*, **52**, 1583–1592.
- Karhunen M, Merilä J, Leinonen T, Cano JM, Ovaskainen O (2013) driftsel: An R package for detecting signals of natural selection in quantitative traits. *Molecular Ecology Resources*, **13**, 746–754.
- Kenney AM (2011) Evolutionary Genetics and Ecology of Water Use Efficiency (13 C) in *Ipomopsis aggregata* and *Arabidopsis thaliana*. University of Texas at Austin.
- Kirkpatrick M (2009) Patterns of quantitative genetic variation in multiple dimensions. *Genetica*, **136**, 271–284.
- Kruuk LEB (2004) Estimating genetic parameters in natural populations using the “animal model.” *Philosophical Transactions B*, **359**, 873.
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution*, **37**, 1210–1226.
- Leinonen T, McCairns RJS, O’Hara RB, Merilä J (2013) Q(ST)-F(ST) comparisons: evolutionary and ecological insights from genomic heterogeneity. *Nature reviews. Genetics*, **14**, 179–90.
- Lotterhos KE, Whitlock MC (2014) Evaluation of demographic history and neutral parameterization on the performance of FST outlier tests. *Molecular Ecology*, **23**, 2178–2192.
- Martin G, Chapuis E, Goudet J (2008) Multivariate Qst-Fst comparisons: a neutrality test for the evolution of the G matrix in structured populations. *Genetics*, **108**, 2135–2149.
- McKay JK, Latta RG (2002) Adaptive population divergence: Markers, QTL and traits. *Trends in Ecology and Evolution*, **17**, 285–291.
- Melendez-Ackerman EJ (1997) Patterns of Color and Nectar Variation Across an *Ipomopsis* (Polemoniaceae) Hybrid Zone. *American Journal of Botany*, **84**, 41.
- Melendez-Ackerman EJ, Campbell DR (1998) Adaptive significance of flower color and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution*, **52**, 1293–1303.
- Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, **14**, 892–903.

- Mitchell RJ (1993) Adaptive significance of *Ipomopsis aggregata* nectar production: observation and experiment in the field. *Evolution*, **47**, 25–35.
- Mitchell RJ (1994) Effects of Floral Traits, Pollinator Visitation, and Plant Size on *Ipomopsis aggregata* Fruit Production. *The American Naturalist*, **143**, 870.
- Mitchell RJ (2004) Heritability of nectar traits: why do we know so little? *Ecology*, **85**, 1527–1533.
- Mitchell RJ, Waser NM (1992) Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. *Ecology*, **73**, 633–638.
- Nuismer SL, Thompson JN, Gomulkiewicz R (1999) Gene flow and geographically structured coevolution. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 605–609.
- O’hara RB, Merilä J (2005) Bias and Precision in QST Estimates: Problems and Some Solutions. *Genetics*, **171**, 1331–1339.
- Ovaskainen O, Karhunen M, Zheng C, Arias JMC, Merilä J (2011) A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. *Genetics*, **189**, 621–632.
- Porter JM, Johnson LA (2000) A phylogenetic classification of Polemoniaceae. *Aliso*, **19**, 55–91.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Provan J, Powell W, Hollingsworth PM (2001) Chloroplast microsatellites: new tools for studies in plant ecology and evolution. *Trends in ecology & evolution*, **16**, 142–147.
- Rogers AR, Harpending HC (1983) Population structure and quantitative characters. *Genetics*, **105**, 985–1002.
- Sahli HF, Conner JK, Shaw FH, Howe S, Lale A (2008) Adaptive Differentiation of Quantitative Traits in the Globally Distributed Weed, Wild Radish (*Raphanus raphanistrum*). *Genetics*, **180**, 945–955.
- Sinervo B, Svensson E (2002) Correlational selection and the evolution of genomic architecture. *Heredity*, **89**, 329–338.
- Spitze K (1993) Population structure in *Daphnia obtusa*: Quantitative genetic and allozymic variation. *Genetics*, **135**, 367–374.
- Stearns F, Boles S, Hurston H *et al.* (2008) Identification of nuclear microsatellite loci for *Ipomopsis aggregata* and the distribution of pairwise relatedness in a natural population. *Molecular Ecology Resources*, **8**, 437–439.
- Stebbins GL (1970) Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307–326.
- Streisfeld MA, Kohn JR (2005) Contrasting patterns of floral and molecular variation across a cline in *Mimulus aurantiacus*. *Evolution; international journal of organic evolution*, **59**, 2548–2559.
- Thompson JN (1994) *The Coevolutionary Process*. The University of Chicago Press, Chicago.
- Thompson JN (1999) Specific Hypotheses on the Geographic Mosaic of Coevolution. *The American Naturalist*, **153**, S1–S14.
- Vitti JJ, Grossman SR, Sabeti PC (2013) Detecting Natural Selection in Genomic Data. *Annu. Rev. Genet.*, **47**, 97–120.
- Wade MJ, Goodnight CJ (1998) Perspective: The Theories of Fisher and Wright in the Context of Metapopulations: When Nature Does Many Small Experiments. *Evolution*, **52**, 1537–

1553.

- Walsh B, Blows MW (2009) Abundant Genetic Variation + Strong Selection = Multivariate Genetic Constraints: A Geometric View of Adaptation. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 41–59.
- Waser N, Chittka L, Price M, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. *Ecology*, **7**, 1043–1060.
- Whitlock MC (1999) Neutral additive genetic variance in a metapopulation. *Genetical research*, **74**, 215–221.
- Whitlock MC (2008) Evolutionary inference from Q ST. *Molecular Ecology*, **17**, 1885–1896.
- Whitlock MC, Guillaume F (2009) Testing for Spatially Divergent Selection: Comparing QST to FST. *Genetics*, **183**, 1055–1063.
- Wilken D, Allard S (1986) Intergradation among Populations of the *Ipomopsis aggregata* Complex in the Colorado Front Range. *Systematic Botany*, **11**, 1–13.
- Wolf PG, Soltis PS (1992) Estimates of gene flow among populations, geographic races, and species in the *Ipomopsis aggregata* complex. *Genetics*, **130**, 639–647.
- Wolf PG, Soltis PS, Soltis DE (1991) Genetic Relationships and Patterns of Allozymic Divergence in the *Ipomopsis aggregata* Complex and Related Species (Polemoniaceae). *American Journal of Botany*, **78**, 515–526.
- Wright S (1951) The Genetical Structure of Populations. *Annals of Eugenics*, **15**, 323–354.

### Data Accessibility

Microsatellite and floral phenotype data available at Dryad (doi: 10.5061/dryad.5c8n1). Custom scripts used for population genetic and multivariate analysis available on github (<https://github.com/ermilano/qstfst>).

### Author Contributions

This manuscript is part of the PhD thesis of E.R.M. T.E.J. and E.R.M conceived the project and undertook field sampling. A.M.K. undertook field sampling and performed greenhouse work.

E.R.M. performed greenhouse work, data analyses, and wrote the manuscript with contributions from all authors.



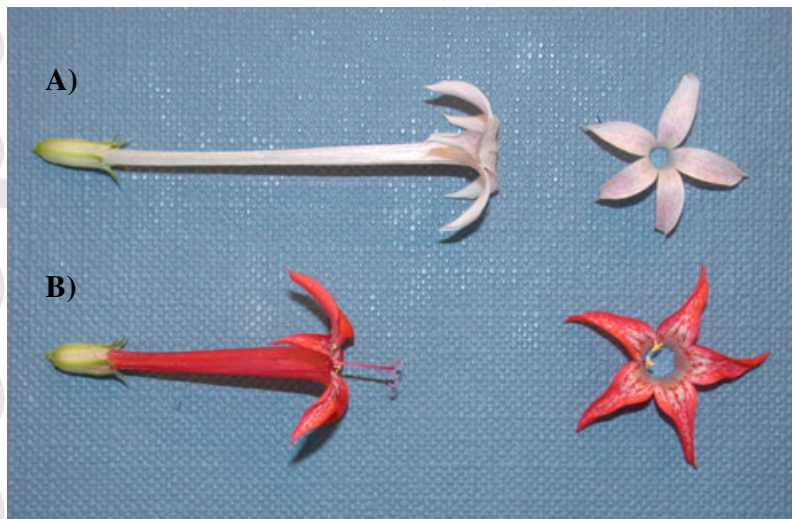
a.  $G_W$

	Corolla Width	Corolla Length	Petal Lobe Length	Color	Nectar Volume	Nectar Concentration
Corolla Width	0.2395					
Corolla Length	-0.0721	0.2500				
Petal Lobe Length	-0.0154	0.0344	0.5915			
Color	-0.0029	-0.0017	0.0445	0.1063		
Nectar Volume	0.0173	-0.0094	0.1163	0.0409	0.3067	
Nectar Concentration	0.0018	-0.0150	-0.1399	-0.0097	-0.1737	0.5987

b.  $G_B$

	Corolla Width	Corolla Length	Petal Lobe Length	Color	Nectar Volume	Nectar Concentration
Corolla Width	0.6987					
Corolla Length	-0.5788	0.5761				
Petal Lobe Length	0.2772	-0.2004	0.1533			
Color	0.5961	-0.6112	0.1169	0.9030		
Nectar Volume	0.6397	-0.5518	0.2381	0.6060	0.5982	
Nectar Concentration	0.0533	-0.0606	0.0255	0.0692	0.0598	-0.0125

**Table 1.** Genetic covariance matrices (a) within and (b) between collection sites. Additive genetic variance for each trait occurs on the diagonal, and genetic covariance along the off-diagonal. We report the initial genetic covariance matrix estimated between populations ( $G_B$ ), before positive-definite matrix correction.



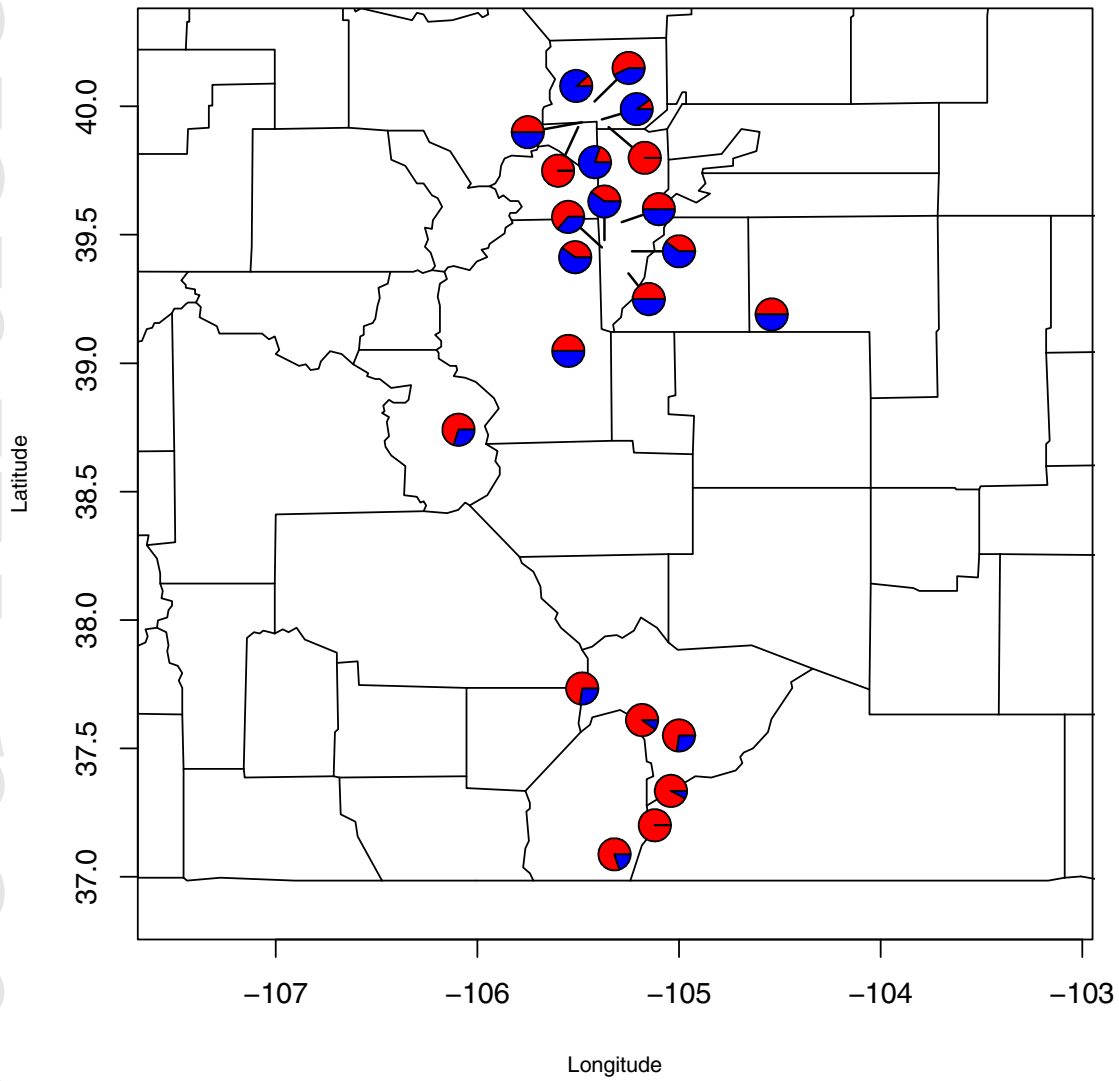
**Figure 1**

Typical (a) *I. a. candida* flowers display long slender white corollas whereas (b) *I. a. collina* flowers display shorter more robust red corollas.



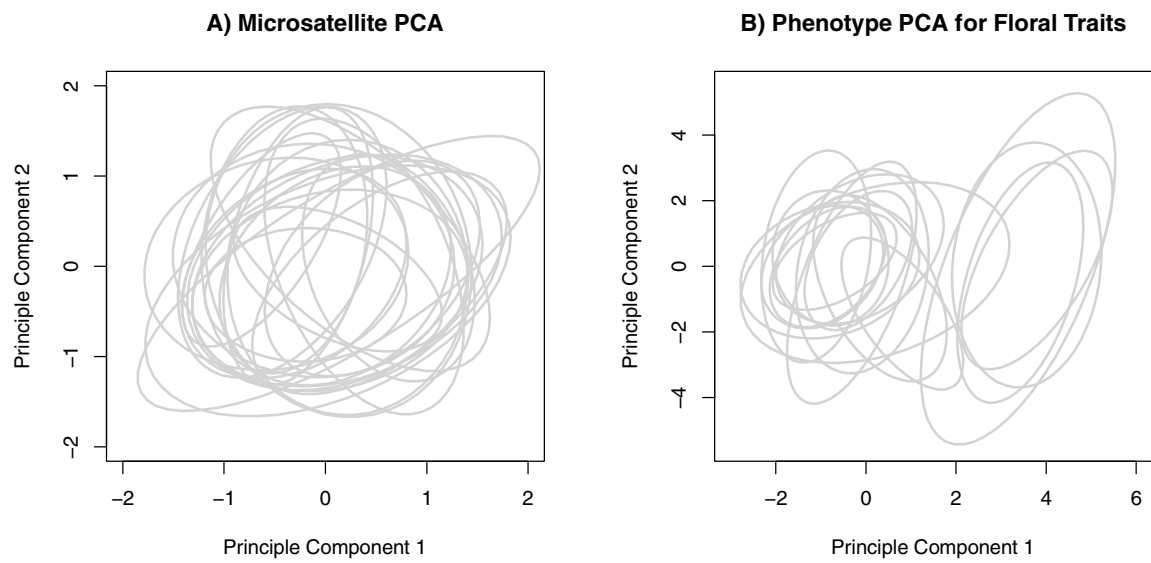
**Figure 2**

Hybrid *I. a. candida* and *I. a. collina* flowers display a spectrum of color and shape combinations as shown in the field (above) and close-up (below).



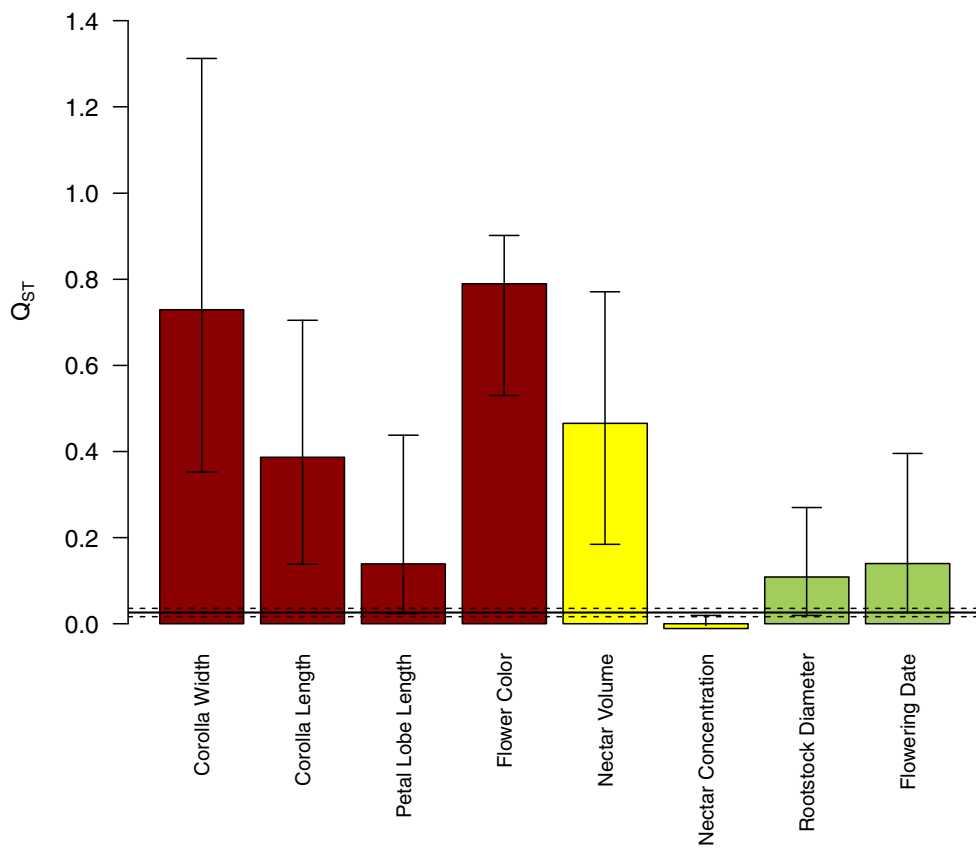
**Figure 3**  
Map of collection sites for neutral markers in the Front Range of the Colorado Rocky Mountains with pie charts indicating cluster assignments from DAPC (K=2).





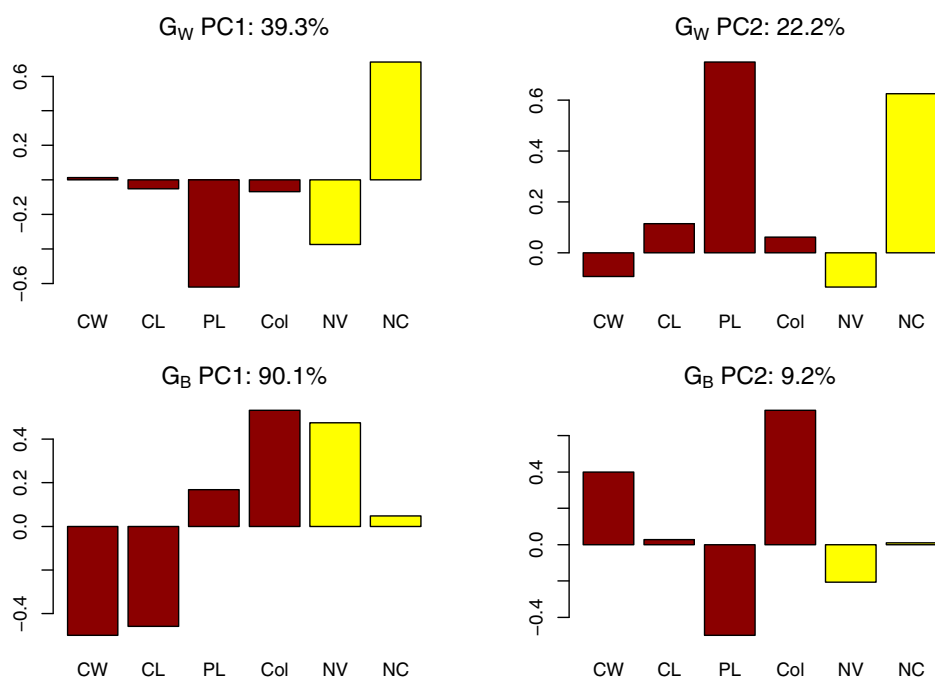
**Figure 4**

Principal components (PCs) 1 and 2 for (a) neutral nuclear microsatellite loci and (b) quantitative floral phenotypes. Dots represent population means, ellipses indicate 95% CI.



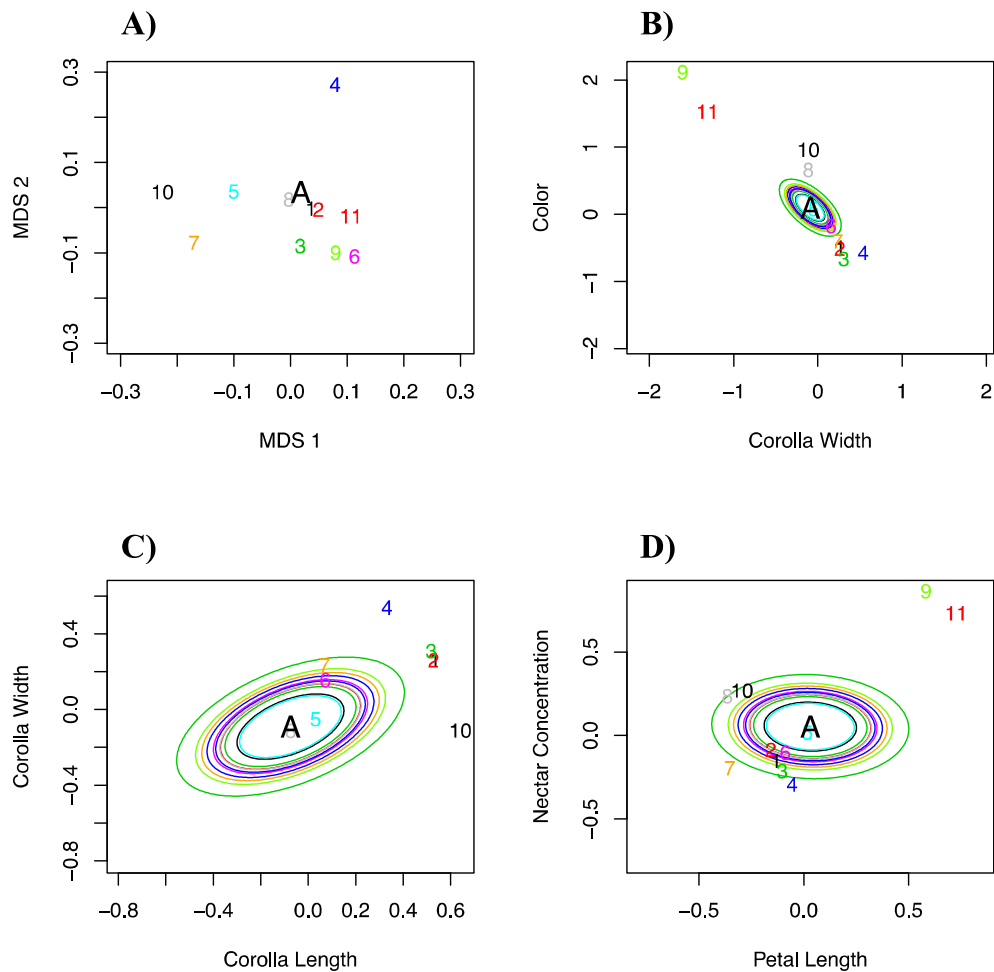
**Figure 5**

Q<sub>ST</sub> and 95% confidence estimates for display (red), reward (yellow), and life history (green) traits. F<sub>ST</sub> (solid) and 95% confidence estimate (dashed) for nuclear microsatellite loci indicated by horizontal lines.



**Figure 6**

Trait loadings, and percent variance explained by the first two principal components (PCs) for the within ( $G_W$ ) and between ( $G_B$ ) genetic covariance matrices. CW= corolla tube width, CL= corolla tube length, PL= petal lobe length, Col= color, NV= nectar volume, and NC=nectar concentration.



### Figure 7

Results from *driftsel* analysis: (a) Neutral genetic distance for each population mean from the inferred ancestor, **A**. Distance calculated as units of ancestral standard deviation and plotted in a 2D plane using multidimensional scaling. Figure produced using modified code from the *viz.theta* function in *driftsel*. (b-d) Additive genetic population means for selected trait pairs, indicated by population ID. Corresponding color ellipses, centered on inferred ancestral mean, represent the median estimated drift distance for each population. Figure produced using modified code from the *viz.traits* function in *driftsel*. Unique population IDs correspond to locations in Table S1.