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Quantitative Genetics and Genotype by Environment Interactions for Flowering Time and Floral Morphology in *Ipomopsis longiflora* subsp. *australis* (Polemoniaceae)

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Quantitative Genetics and Genotype by Environment Interactions for Flowering Time and Floral Morphology in *Ipomopsis longiflora* subsp. *australis* (Polemoniaceae)

by

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Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Arts

The University of Texas at Austin

May 2015

Acknowledgements

I would like to thank the all the people who have helped to contribute to this work either directly through their participation in this research or indirectly through the support they have given me throughout this long and draining process. This research project would not have been possible without the support of Thomas E. Juenger, or funding from the Department of Integrative Biology. I would also like to thank my family for helping me through the tough times and reading numerous manuscripts. Finally, none of this would have been possible without the love and understanding of my wonderful wife Stavana Strutz who has helped me wade through the murky waters down the home stretch.

Abstract

Quantitative Genetics and Genotype by Environment Interactions for Flowering Time and Floral Morphology in *Ipomopsis longiflora* subsp. *australis* (Polemoniaceae)

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The University of Texas at Austin, 2015

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Flowering phenology and floral morphology are both directly tied to overall reproductive success of flowering plants. The match between pollinator abundance and timing of flowering can greatly affect plant fitness, and flower shape and size affect attractiveness of plants to pollinators. I measured quantitative genetic parameters for flowering time (date of first flower) and floral morphology in a polycarpic desert annual, *Ipomopsis longiflora* subsp. *australis* to determine the potential for these traits to respond to selection. Significant heritabilities and coefficients of genetic variation (CV_A) were found for flowering phenology and most of the floral traits measured, indicating these traits can likely respond to natural selection in natural populations. Although significant genetic correlations were calculated between many of the floral characters to assess possible constraints on floral evolution, none were detected between flowering time and floral morphology. Flowering time did have a significant genotype-by-environment interaction (GxE) in response to greenhouse and field growing conditions, indicating that there is

genetic variation in plasticity for flowering time in *Ipomopsis longiflora*. Plasticity in flowering time may be adaptive in *Ipomopsis longiflora* due to temporally varying selection pressures associated with differing growing and reproductive seasons faced in the desert southwest.

Table of Contents

List of Tables

List of Figures

Chapter 1: Quantitative Genetics of Phenology and Floral Morphology

INTRODUCTION

Flowering time and floral morphology have large effects on fitness in angiosperms. Even small differences in first flowering date can have large fitness consequences (Fox, 1989b; Kelly and Levin, 1997; Stinson, 2004). Selection on flowering time contributes to local adaptation in a number of species including *Mimulus guttatus* (Hall and Willis, 2006), wild barley (Verhoeven et al., 2008), *Arabidopsis thaliana* (Stinchcombe et al., 2004; Shindo et al., 2005; Li et al., 2006; Izawa, 2007), rice (Izawa, 2007), and *Populus balsamifera* (Keller et al., 2012). Synchronization of flowering time with pollinator availability is particularly crucial to reproductive success (O'Neil, 1999; Thomson, 2010). Floral morphology affects pollinator preference and visitation and thereby fitness. Numerous studies have demonstrated pollinator-mediated selection on floral morphology, including corolla shape (Herrera, 1993; Nattero et al., 2010; Kaczorowski et al., 2012), corolla size (Campbell et al., 1996; Conner et al., 1996), stigma position (Campbell et al., 1994), as well as flowering time (Johnston, 1991).

Evolutionary change in flowering time and floral morphology cannot occur without heritable genetic variation. Heritability (h^2) of flowering time has been estimated for a wide range of species, and has usually been found to be low (mean h^2 for phenology = 0.20, Geber and Griffen, 2003). When heritability for flowering time is estimated in the field versus the greenhouse (Weinig et al., 2002), heritability estimates in the field tend to be lower, on average, than in the greenhouse for most traits (Geber and Griffen, 2003). The low heritabilities may be due to plasticity in flowering time, and the plasticity itself may be adaptive under variable environmental conditions.

Heritability has also been estimated for floral morphology in a number of species. On average, intermediate h^2s have been estimated for floral traits involved in pollinator attraction and mating system (averages of 0.46 and 0.41 respectively, Ashman and Majetic, 2006). These values indicate that floral morphology is often able to respond to selection. Heritability of floral morphology has been estimated in both field and greenhouse for Raphanus (Conner et al., 2003). Conner et al. only found significant h^2 for some traits in the field while all traits were significant in the greenhouse and values differed between environments.

Flowering time and floral morphology tend to be plastic, with genotypes exhibiting different phenotypes depending on the environment (G×E). For example, flowering time differences in *Eriogonum abertianum* have been shown to be almost entirely due to plastic responses to water availability (Fox, 1989a). GxE can be interpreted as genetic variation for phenotypic plasticity, and it can alter the rate and direction of response to selection (Via and Lande, 1985). Therefore GxE can affect estimates of heritability and responses to selection.

Genetic correlations influence responses to selection, affecting rates and causing correlated responses. Phenotypic and genetic correlations have been found frequently among floral characters, with characters involved in attraction of pollinators and mating system often positively correlated (average r_g= 0.32, Ashman and Majetic, 2006). Pleiotropy, a single locus affecting multiple phenotypes, has often been found to be the cause of genetic correlations in flowers (Conner, 2002; Juenger et al., 2005; Hall et al., 2006; Edwards and Weinig, 2011). However, assortative mating can also result in genetic correlations via linkage disequilibrium. Phenological assortative mating paired with pollinator mediated selection could result in the evolution of correlations between floral

characters and flowering time, e.g. changes in pollinator types through time (Weis and Kossler, 2004).

Ipomopsis longiflora (Torr.) V. Grant subsp. *australis* Fletcher & W. L. Wagner (Fletcher and Wagner, 1984), is a desert annual that experiences temporally varying selection on flowering time (LeBuhn, 1998). LeBuhn found a complex pattern of strong phenotypic selection on flowering time that varied across seasons and years. LeBuhn also documented differences in pollinator communities between spring and fall flowering seasons. The spring season was dominated by Hawkmoths (primarily *Hyles lineata*), while bees made up 27% of the visitors in the fall (LeBuhn, 1998, 2004). Despite detailed ecological data, no information on the genetic basis of flowering time or floral morphology is available for *I. longiflora.* As such, the evolutionary effects of life history plasticity and varying pollinator communities on *I. longiflora* have never been documented.

In this study, a large common garden experiment was conducted to estimate heritabilities and genetic correlations of flowering time and floral morphology in *I. longiflora*. The experiment was conducted in both greenhouse and field conditions to examine the effects of environmental variation on flowering time and floral morphology. The goals of this study were to (1) determine whether flowering time (first flowering date) and floral morphology have significant heritable variation, (2) determine whether there are any significant phenotypic or genetic correlations for flowering time and floral morphology, (3) determine whether correlations between flowering time and floral morphology are consistent with the hypothesis of selection on floral morphology by temporally varying pollinator communities and (4) determine whether there are significant environmental or GxE effects on flowering time and floral morphology.

MATERIALS AND METHODS

Study Species

Ipomopsis longiflora subsp. *australis* is a desert annual found in the Chihuahuan and Sonoran Deserts (Fletcher and Wagner, 1984). Populations near Portal, AZ, flower in both the spring (April – May) and fall (September – October) (LeBuhn, 1998, 2004). *I. longiflora* is a facultative outcrosser with long, tubular, primarily white flowers, which are primarily pollinated by the white-lined sphinx moth *Hyles lineata* (LeBuhn, 1998, 2004). Effective outcrossing rates in the field have yet to be measured, but pollinator visitation is common (LeBuhn, 1998, 2004). Therefore, outcrossing rates in the field are expected to be high despite high glasshouse selfing rates (personal observation).

Plant Material and Experimental Design

Ipomopsis longiflora is typically found in marginal or disturbed habitats like roadsides or washes. Seed collection was conducted at five sites across the Chihuahuan Desert (Fig. 1, supplemental Table s1), all of which were roadsides near Portal, AZ. I included the site used by Lebuhn (1998, 2004). Every reproductive individual was sampled at each collection site, from 8 to greater than 50 individuals per site, and multiple fruits were collected from each individual when possible for a total of 160 individuals. All seeds were collected on a maternal plant basis, and each plant usually contributed > 20 seeds. Seeds were collected in both spring and fall flowering seasons between May 11, 2009 and November 21, 2009.

Experimental plants were germinated from seeds collected from a total of 160 maternal families. Seeds were sown in 2.5" pots on sand and cold stratified in the dark at 5° C for 6 weeks. Germinated seedlings were transferred to the greenhouse for two weeks then transplanted to SC10 Super Ray Leach "Cone-tainers." Greenhouse conditions

consisted of supplemental light for 16 hour days and maximum daytime temperatures of 29.4° C. Initially, ten plants per family (1600 total) were planted but due to mortality 1263 individuals (7.9 ± 2.3 plants per family) were used for data collection.

Maternal families were split evenly between greenhouse and field-nursery growing environments once they reached the rosette stage (approximately 4 weeks) if they had greater than four seedlings ($n_{nursery}$ = 123). Families with fewer than four seedlings were only assigned to the greenhouse ($n_{greenhouse}$=160). The plants assigned to the nursery environment were moved outdoors on May, 01, 2010. In both greenhouse and nursery environments, plants were grown on tables, in racks of 10 plants. Seedlings from each family were first divided across tables and then randomized into racks.

The growing conditions consisted of glasshouse with supplemental light for 16 hour days and max daytime temperature of 29.4°C (greenhouse), and tables arranged in a field-nursery at Brackenridge Field Laboratory at UT-Austin (nursery). Nursery plants experienced temperatures ranging from 12.2°C to 37.2°C with average highs of 33.9°C and day lengths from 13 hr 22 min to 14 hr 06 min. Plants in both environments were watered every other day with low-dose micro-nutrient fertilizer water (Dyna-Gro Liquid Grow 1:100 injector ratio). Photoperiod and daytime temperatures in the nursery environment were similar to those that would be experienced by spring (April – May) flowering *Ipomopsis longiflora* in AZ, while greenhouse day lengths were much longer than natural. Other variables that were not measured likely differed between the two environments e.g. humidity, soil moisture, and light intensity.

Methods and Data Collection

Plants were monitored daily, date of first flowering was recorded. Flowering time (first flowering date) was the number of days after plants were split between environments

until anthesis. Because plants were transplanted across several days, the number of days from germination to transplant was also calculated and used as a covariate in subsequent analyses. This period of time was included to control for effects of germination time and environment prior to exposure to experimental environments.

Floral morphology was measured on a subset of the total experimental population. Flowers were measured on 739 plants, on a total of 1043 flowers, from 154 maternal lines (4.8 ± 2.0 plants per maternal line). This subset represent nearly every maternal line, with slightly reduced sample sizes per line across the entire flowering period. Sampling was largely restricted due the strict selection criteria for floral measurement, flowers were carefully selected so they were similar ages and at the same stage of development. All flowers were measured using digital calipers on the first day of anthesis, once stigma lobes had reflexed. Floral traits measured were: corolla tube length, corolla tube width at the flower opening, stigma length, sepal length, anther-stigma distance (ASD), anther exertion, petal length, petal width, and the angle of petal reflexion (Figure 2). Stigma length was measured as the length of the entire pistil, and anther exertion was the distance the longest anther was exerted from the corolla tube. The angle of reflexion of the petals from the face of the flower was used because this measure is independent of the size of the petal and affects the amount of petal visible to pollinators. Reflex angle was calculated as arcsine (opposite/hypotenuse), where opposite = depth of reflex and hypotenuse=petal width, and depth of reflex is equal to the distance the petal reflexed away from the opening of the corolla tube (Fig. 2C). Anther-stigma distance (ASD) was calculated as: ASD = stigma length – (corolla length + anther exertion) (see Figure 2). ASD is a measure of herkogamy, or the distance between anthers and stigma (at sexual maturity), and is often highly correlated with the rate of autogamous selfing (Ennos, 1981; Motten and Stone, 2000; Arathi and Kelly, 2004).

Statistical analyses were performed using mixed models with PROC MIXED in SAS software (version 9.3) to explore the impact of fixed and random effects on floral morphology and flowering time (Fry, 2004; SAS Institute Inc., 2012). The full model consisted of fixed effects: collection location (Population), growing environment (Environment), collection season (Season), and the covariate number of days between germination and transplant (Days to Transplant); and random effects: maternal family nested within Population (Family), the interaction of maternal family within Population and Environment (GxE), large scale blocking (Table), and rack within Table (Rack). A model-fitting phase was performed for each trait independently. This phase consisted of 1. fitting the full model; 2. removing fixed effects that were not significant; 3. conducting likelihood ratio tests (LRT) for random effects (Fry, 2004); and 4. dropping random effects not significantly different than zero (excluding Family). This strategy was taken to avoid over-parameterization and because I had no *a priori* hypotheses about certain effects. Population and Environment were included in every model, but Season and Days to Transplant were dropped from the models where they were not significant. Season was initially included in the model to account for differences between seeds collected in different seasons. Family was also included in every model because it was necessary to estimate genetic variance. Likelihood ratio tests were used to determine whether variance components for random effects were significantly greater than zero (Fry, 2004).

GxE by can be driven by a number of different factors, including differences in among or within genotype variances in each environment or by rank changing of genotypes in each environment. For traits for which GxE was significant, a second model fitting step was conducted using LRT tests in PROC MIXED in SAS (SAS Institute Inc., 2012) to test for significant differences within and/or between genotype variances across environments (Fry, 2004). The full model for traits with significant GxE was expanded in several ways:

first, separate within-genotype (residual) variances were estimated for each environment by setting Environment as the group using a 'repeated' statement; next, separate among genotype variances for each environment were estimated by using a separate 'random' statement with the unstructured correlations (UNR) covariance structure specified. Changing the covariance structure to UNR also estimates the genetic correlation between families across environments. Finally, LRT were used to test whether the among- and within-genotype variances differed across environments as well as the significance of the genetic correlation.

The variance components estimated by the mixed model were used to calculate heritability and the additive genetic coefficient of variation (CV_A). The variance components estimated by the mixed model were: V_{Fam} = variance among maternal families and V_P = the total phenotypic variance (sum of all variance components: V_{Table}, V_{Rack}, V_{Fam}, V_{GxE}, $V_{residual}$; see Table 1 for each trait). Heritability was calculated in the following ways due to the unknown paternity of seeds and the potential for selfing: half-siblings $h^2_{(half)} = 4\ V_{Fam}/V_P$; full-sibling families $H^2_{(full)} = 2\ V_{Fam}/V_P$; and selfed seed $H^2 = V_{Fam}/V_P$ (Falconer and Mackay, 1996). These different calculations scale the genetic variance based on relationship of the individuals in each family and since the true relationship is unknown all three possibilities are calculated. Full-sibling and selfed seeds calculations cannot separate non-additive genetic variance such as dominance (V_D) and maternal effects making these broad-sense heritabilities (Falconer and Mackay, 1996). Standard errors were calculated for heritability using the delta method (Lynch and Walsh, 1998). The additive genetic coefficient of variation was calculated, as $CV_A = 100\sqrt{}\ V_A/\bar{X}$, were $\bar{X}$ = mean, and $V_A = 4V_{Fam}$ (Houle, 1992).

Phenotypic and genetic correlations were estimated using JMP Genomics 7 (SAS Institute Inc., 2014). Pearson product-moment correlations were calculated among

family breeding values for genetic correlations. Breeding values were Estimated Best Linear Unbiased Predictions (EBLUPs) for Family calculated from the mixed model in SAS (SAS Institute Inc., 2012). Pearson product-moment correlations were calculated for phenotypic correlations using the entire data set. P-values were corrected for multiple tests using the false discovery rate (FDR) (Benjamini and Yekutieli, 2001).

RESULTS

Heritability and Coefficients of Variation

In the Linear Mixed Models, the fixed effects Days-to-Transplant and Season had no influence on most of the traits, with the exception of significant Transplant effects on reflex angle and flowering time, and Season effects on petal length and sepal length (Table 1). There was a significant Environment effect for all morphological characters except anther exertion, and petal length and width (Table 1). Flowering time was not significantly different between environments (Table 1). Populations also differed in most of the floral characters excluding anther exertion, corolla width, and reflex angle. Interestingly, flowering time differed strongly between populations even though some were located only a few miles from each other (Table 1, s1).

Significant genetic variance was discovered for all the traits except sepal length and corolla width (Table 1). Heritabilities ranged from 0.233 ± 0.094 to 0.760 ± 0.146 when calculated using half-sibling families, while H^2 were much smaller for selfed seed, from 0.058 ± 0.024 to 0.190 ± 0.037 (Table 2). Full-sibling values were intermediate and may be more representative of mixed seed collection, so they are presented. The highest $H^2_{(full)}$ was for petal width (0.38 +/- 0.073), while ASD (0.311 +/- 0.074), corolla length (0.309 +/- 0.074), and stigma length (0.311 +/- 0.073) were of similar magnitude (Table 2). Due to significant GxE for flowering time, separate within-family variance

components were estimated in each environment and H^2 and CV_A were calculated separately for both environments. $H^2_{(full)}$ for flowering time was about twice as large inside the greenhouse (0.222 +/- 0.09) as the nursery (0.116 +/- 0.047) due to lower environmental variance (Table 2). Table was only significant for flowering time and petal length, while Rack was significant for the majority of the traits (Table 1) suggesting relatively fine scale environmental variation in both the greenhouse and field nursery.

Sepal length and corolla width had the lowest CV_As (4.96 and 0.56 respectively). ASD had the highest CV_A (205.721) which was nearly an order of magnitude greater than the next highest value (flowering time (greenhouse): 32.079). CV_A values for the rest of the traits were between 6.65 and 27.52, which are similar to values previously reported for floral characters (Juenger et al., 2000, 2005; Conner et al., 2003; Kaczorowski et al., 2008). Interestingly, anther exertion and reflex angle had large CV_A values (>15) even though they had lower heritabilities (Table 2). This indicates these traits have higher potential evolvabilities than the other morphological traits with larger heritabilities. CV_A for flowering time was calculated for both environments and was quite high in both despite differences in heritability (Table 2).

Phenotypic and Genetic Correlations

Significant phenotypic correlations were present among most of the morphological characters (Table 3), and of the 22 significant correlations, only 3 were negative (Table 3). The strongest positive correlation was between stigma length and corolla length (0.733) while the rest of the positive correlations were much weaker (0.138 to 0.492). The strongest negative correlations were between ASD and two of its components, corolla length (-0.335) and anther exertion (-0.240). There was also a weak negative phenotypic correlation

between flowering time and anther exertion (-0.089), but this was the only significant correlation with flowering time.

All of the genetic correlations were similar in magnitude and in the same direction as the phenotypic correlations (Table 3), but fewer were statistically significant (14). Flowering time was not genetically correlated with any of the morphological characters despite evidence that selection on floral morphology may vary temporally in *Ipomopsis longiflora* (LeBuhn, 1998). Like the phenotypic correlations, there were only 3 negative genetic correlations. Also similar, stigma length and corolla length had the strongest genetic correlation (0.715), indicating that the phenotypic correlation has a genetic basis. Once again, ASD was negatively correlated with corolla length (-0.310) and anther exertion (-0.270), which may be due to their functional relationship. Decreases in corolla length (without corresponding changes in either anther or stigma position) will necessarily cause an increase in ASD. However, the strongest negative genetic correlation observed was between petal width and reflex angle (-0.535), which are both related to the area of petal visible to pollinators.

Genotype by Environment Interactions

There was a significant GxE term for flowering time but not floral morphology (Table 1). There was a significant across environment genetic correlation for flowering time, $r_A = 0.47$ (LRT: $r_A > 0$: $\chi^2 = 6.3$, df = 1, p = 0.006; $r_A < 1$: $\chi^2 = 8$, df = 1, p = 0.0023), with a pattern of most families flowering earlier in the greenhouse (Fig. 3). Residual variances for flowering time significantly differed across environments (LRT: $\chi^2 = 79$, df = 1, $p < 0.0001$, Table 1), resulting in greater V_E in the nursery. Among-family variances for flowering time were not significantly different across environments (LRT: $\chi^2 = 0.5$, df = 1, p = 0.24), indicating that changes in V_G were not the cause of the GxE but rather was

the result of rank changing of families across environments. Interestingly, family means showed extensive rank changing between environments (Fig. 3).

DISCUSSION

Heritabilities and CV_A

One of the goals of this study was to determine quantitative genetic parameters for flowering time and floral morphology in *Ipomopsis longiflora*. Flowering time and floral morphology are both potentially adaptive in *I. longiflora* and contain considerable variation in natural populations. Quantitative genetic parameters such as H^2 and CV_A can help determine how evolution may occur in response to selection in *I. longiflora*.

In the univariate case, increased narrow-sense heritability improves the response to selection (R) for a given selection differential (S) (breeders' eqn: $R = h^2*S$). Most of the traits measured exhibited substantial heritable genetic variation, which would allow for a response to selection. However, multivariate interactions, including the selection gradient and G-matrix antagonistic correlations might cause genetic constraints opposing selection (Conner and Via, 1993; Falconer and Mackay, 1996). While selection was not measured in this study or applied to particular traits, previous work in this species and in the south foothills population (Fig. 1, Table s1) can indicate how selection might be acting (LeBuhn, 1998). LeBuhn calculated linear selection differentials (s) and gradients (β) and found selection on first flowering date at both fine grained (within generation) and coarse grained (across generation) temporal scales. For fine-grain selection, double-flowering plants were measured across seasons ($Spring_{1994}$: s = -1.2; $Fall_{1994}$: s = -1.65, $Fall_{1995}$: β = 0.3 +/- 0.15) and for coarse grained selection single flowering plants were compared across years ($Fall_{1994}$: s = 1.30). Significant heritabilities for first flowering date were estimated using plants from the same population (H^2_{full} greenhouse = 0.22, H^2_{full} nursery = 0.11, Table 2),

indicating relatively strong responses to selection are possible. LeBuhn's estimates demonstrated that the direction and magnitude of selection on flowering time changed from season to season and year to year in the south foothills population. The temporally varying selection on time to flowering could be responsible for maintaining the quantitative genetic variation present for flowering time (Burger and Gimelfarb, 2002).

The majority of the floral traits measured also had significant heritability, including anther length, anther stigma distance (ASD), corolla length, petal width, reflex angle, and stigma length (Table 2). Heritabilities for these traits were within the range of heritabilities previously reported for floral morphology (Good-Avila and Stephenson, 2002; Ashman and Majetic, 2006; Hansen et al., 2011). CV_A, which allows comparisons across traits and predicts evolvability, was also high for many of the floral traits, indicating that these traits can respond to selection or evolve (Houle, 1992; Hansen et al., 2011).

Heritability was calculated for maternal families assuming they were half-sibling, full-sibling, or selfed seed. This was due to the unknown parentage and the mixed mating system (facultative outcrossing). However, due to observations of frequent pollinator visitation in the field (LeBuhn 1998, 2004, personal observation), and the use of multiple fruits collected from each plant, it is likely that some if not all the families are actually half-siblings. Since heritability equations for full-sib families and selfed seed result in smaller values, it is likely that true heritability is somewhere between the half-sib and selfed values (Falconer and Mackay, 1996).

The primary pollinator of *Ipomopsis longiflora* is the medium sized hawkmoth, *Hyles lineata* (Sphingidae) (LeBuhn, 1998, 2004). While pollinator preferences have not been measured in *I. longiflora*, hawkmoths generally prefer longer corolla length and decreased corolla width in congeneric species, including I. *aggregata* and *I. tenuituba* hybrid zones (Campbell et al., 1997; Aldridge and Campbell, 2007). Corolla width had no

significant heritability or CV_A (Table 2). Strong selection by hawkmoths and subsequently evolutionary responses could have reduced variation in corolla width (Fisher, 1930; Aldridge and Campbell, 2007). The clade containing *I. longiflora* also has hummingbird- (*I. sancti-spiritus*, *I. aggregata* ssp. *bridgesii*) and bee-pollinated species (*I. multiflora multiflora*, *I. polyantha*) (Porter et al., 2010). Therefore the evolution of hawkmoth pollination in *I. longiflora* may have involved responses to selection on corolla width to exclude other pollinators as in other *Ipomopsis* species (Campbell et al., 1997; Aldridge and Campbell, 2007). Hawkmoths also have preferences for flower shape. Both Herrera and Kaczorowski found that hawkmoths prefer more dissected corolla limbs with larger surface area (Herrera, 1993; Kaczorowski et al., 2012). Flower shape and size in *I. longiflora* is determined by petal length and width along with the petal reflex angle, all of which were found to have heritable genetic variation in this population, making these potential targets of selection.

Pollinator communities in *I. longiflora* exhibit seasonal differences from almost exclusively hawkmoth visitation in the spring to nearly 30% bee visitation (both solitary and honey bees) in the fall (LeBuhn, 1998, 2004). Hawkmoths generally prefer larger flowers, with longer corolla tubes and greater surface area, while bees prefer larger overall floral display size (Thompson, 2001). Life history theory suggests that there should be tradeoffs between reproductive traits such as flower size and number based on resource allocation which could result in opposing selection. Interestingly, LeBuhn found no such tradeoffs in *I. longiflora*, with double flowering plants having both larger flowers and more flowers, despite controlling for size (LeBuhn, 2004). Temporally varying selection on different characters or in opposing directions by these different pollinators could either lead to divergence between seasons or more likely maintenance of genetic variation in these traits (Burger and Gimelfarb, 2002).

Mating system characters are also often targets of selection since they are directly related to fitness. While *I. longiflora* is a facultative outcrosser, its sister species *I. laxiflora* is an autogamously selfing species, making ASD and other mating system characters potential targets of selection (Juenger and Bergelson, 2002; Porter et al., 2010). Another indication that ASD may be under selection is that the *Ipomopsis* genus contains variation in self-compatibility, with *I. aggregata* having late-acting ovarian self-incompatibility (Sage et al., 2006). ASD is often highly correlated with the rate of self-pollination and fitness (Barrett, 2003). In this study ASD had the highest CV_A by an order of magnitude (145.46) and one of the highest heritabilities (0.311). This indicates a potential for selection on ASD and other mating system characters in *I. longiflora* and a potential for these traits to respond to those selective pressures.

Genetic Correlations

While floral traits may be targets of selection by pollinators, genetic correlations between these floral traits also influence the response to selection. Genetic correlations between traits are often thought of as constraints to evolution, but this is dependent on the direction of selection (Conner and Via, 1993; Conner, 2012). The majority of the floral size traits such as corolla length and petal length were positively correlated with the other morphological characters in *Ipomopsis longiflora*, although most of the correlates were rather weak (< 0.3, Table 3). Therefore, the evolution of floral shape is somewhat constrained in certain dimensions. Response to selection on overall floral size, either smaller or larger, is possible but single floral traits would not be able to respond to selection independently. For example selection for larger petals on a short flower. Many of the floral organs are integrated in *I. longiflora*, because the corolla is fused into a tube with the filaments fused along the interior, indicating that pleiotropy between floral organs may be

the cause of the correlations. These genetic correlations also limit how display traits such as petal size can evolve. For example, petal size is limited by selection on corolla length since corolla length is often under strong selection by hawkmoths, and limits which pollinators can visit the flower (Herrera, 1993; Ippolito et al., 2004). However, in the fall season the genetic correlations may affect the response to selection differently, since bees prefer greater number of flowers not individual flower length or width selection on size may be relaxed (Thompson, 2001).

The strongest genetic correlation detected was between corolla length and stigma length (0.71), which may have resulted from strong selection on stigma position or pleiotropic effects of genes driving corolla tube and stigma growth. Correlational selection on corolla and stigma length would lead to genetic correlations through a buildup of linkage disequilibrium (Lande, 1984; Brodie III, 1992). Since stigma position is critical to insure outcrossing, independent changes in either corolla or stigma length would result in reduced fitness due to reduced pollen deposition.

A significant positive genetic correlation was also found between corolla width and petal width. Increased petal width is favored by hawkmoths and contributes to overall corolla size and corolla limb shape (Herrera, 1993; Kaczorowski et al., 2012). Smaller corolla tube width is also favored by hawkmoths in other species of plants including other *Ipomopsis* species (Campbell et al., 1991; Caruso, 2001; Campbell, 2003). Therefore directional selection by hawkmoths would be predicted to be for larger petal width and narrower corolla tubes. The positive genetic correlation between petal width and corolla tube width may slow the evolution of hawkmoth preferred flowers.

The majority of the significant genetic correlations were positive with the major exception being a relatively strong negative correlation between petal width and reflex angle (-0.54). These two traits work together to influence pollinator attraction, with

increases in petal width increasing the overall petal area while greater reflex angles decrease the visible petal area for pollinators. Negative genetic correlations can reduce the effectiveness of directional selection, but in this case the negative correlation is presumably along the axis of selection by hawkmoths, which prefer larger surface areas with more petal showing (Herrera, 1993; Kaczorowski et al., 2012).

ASD had negative genetic correlations with both anther length and corolla length and was positively correlated with stigma length in *Ipomopsis longiflora*. Characters influencing mating system are often functionally integrated and also genetically correlated (Ashman and Majetic, 2006). The genetic correlations between mating system traits could be largely functional since decreases in anther and corolla length would cause increases in ASD, and increases in stigma length would decrease ASD. In many self-pollinating species ASD changes during floral development, increasing over time, or is polymorphic (Barrett, 2003). In *I. longiflora* the stigma is originally positioned behind the anthers in the bud. As the flower develops, the stigma usually extends past the anthers before becoming receptive (personal observation), although there is variation in final stigma length allowing for both positive and negative ASD values. Since an ASD value of 0 would almost assure self-pollination, selection for increased selfing rate might be more similar to stabilizing selection than directional selection, with intermediate ASD values having higher fitness than either extreme. Due to the genetic correlations between ASD and anther, corolla, and stigma length, stabilizing selection on ASD potentially leads to indirect or correlational selection on floral morphology (Campbell et al., 1994; Brock and Weinig, 2007).

Interestingly, flowering time was not genetically correlated with any of the traits measured, indicating that the floral traits can respond to selection independent of flowering time. Since pollinator communities vary through time (LeBuhn, 1998, 2004), flowering time should be correlated with any floral traits preferred by each group. The lack of genetic

correlations indicates that alleles causing variation in floral morphology are not the same as those causing variation in flowering time and do not appear to be in linkage or linkage disequilibrium with each other. So while pollinator communities vary temporally and there is evidence of temporally varying selection on flowering time, no evidence was found that floral traits were associated with this pattern of selection.

Genotype by Environment Interaction

Populations of *I. longiflora* experience environmental variation at multiple temporal scales, with both single and double flowering individuals in two seasons. The opportunity for GxE to evolve may influence the ability of traits to respond to selection and is thought to help maintain variation (Via and Lande, 1985).

There was significant GxE for flowering time in this experiment and evidence for genetic variation in plasticity. The correlation between genotypes across the different environments was significant but less than one, indicating that there is genetic variation in the plastic response to the environmental conditions. This allows for plasticity in flowering time to evolve, given that there is selection for plasticity (Price et al., 2003; Pigliucci, 2005; Ghalambor et al., 2007). However, for plasticity to evolve the fitness of plastic genotypes must be higher in both environments, i.e. increase overall fitness (Via and Lande, 1985; van Kleunen and Fischer, 2005), in this case both seasons. Due to the temporally varying pattern of selection in *Ipomopsis longiflora*, plasticity in flowering may also be under selection.

Despite significant GxE for flowering time, the differences between the two environments were not strictly controlled in this experiment. However, two major factors that are known to affect flowering time in numerous species differed between the growing conditions experienced by the experimental plants: day length and temperature (Sourdille

et al., 2000; Shindo et al., 2002; Riihimaki and Savolainen, 2004; Nakagawa et al., 2005; Engelmann and Purugganan, 2006). Glasshouse conditions resulted in longer days (16 hours of light), which exceeds the natural maximum day length for these populations, and regulated daytime temperatures via evaporative coolers. Nursery conditions mimicked natural spring season conditions for *I. longiflora* subsp. *australis* fairly well in terms of day length and temperature, although daily temperature fluctuations are less extreme in Austin, where it is not as cold at night. Interestingly, work controlling both temperature and day length in *Brassica rapa*, found that most of the GxE for flowering time was due to temperature (Edwards and Weinig, 2011). In the *I. longiflora* populations, flowering seasons vary in both day length and temperature, with average spring temperatures in April (high: 21.9°C, low: 1.7°C) and May (high: 26.5°C, low: 5.5°C) and day lengths (12 hr 28 min to 14 hr 02 min) and fall average temperatures in September (high: 26.5°C, low: 9.3°C) and October (high: 22.3°C, low: 3.9°C) and day lengths (12 hr 45 min to 10 hr 53 min). Because the seasons are mirror images of each other, flowering is initiated under drastically different conditions. Temperature varies from year to year and between flowering seasons, and day length varies between seasons, indicating that there may be selection on the plasticity observed for flowering time.

Conclusion

Flowering time and floral morphology were found to have significant heritable genetic variation in *I. longiflora*, allowing for responses to selection. In addition, positive genetic correlations between the majority of the floral traits could promote response to selection for flower size, but also limit independent responses of flower traits. Flowering time showed GxE between the greenhouse and field nursery conditions, potentially caused by differing temperatures or day-lengths between environments. The GxE effect was

characterized by rank changing genotypes, and indicates genetic variation in plasticity for flowering time.

TABLES

Trait	Fixed				Random				
	Environment	Population	Days to Transplant [@]	Season [@]	Family	Rack [@]	Table [@]	G x E [@]	Residual
Anther exertion	NS	NS	NS	NS	0.048 *	0.080 ***	NS	NS	0.652
ASD	*	*	NS	NS	1.914 ****	NS	NS	NS	10.401
Corolla Length	****	*	NS	NS	3.123 **	1.020 *	NS	NS	16.098
Corolla Width	***	NS	NS	NS	2.1×10^{-5} NS	NS	NS	NS	0.097
Petal Length	NS	**	NS	**	0.137 **	0.215 ****	0.070 *	NS	1.587
Petal Width	NS	**	NS	NS	0.267 ***	0.187 ****	NS	NS	0.949
Reflex Angle	****	NS	**	NS	0.004 **	NS	NS	NS	0.044
Stigma Length	**	*	NS	NS	3.482 **	1.393 **	NS	NS	17.522
Sepal Length	****	**	NS	**	0.040 NS	NS	NS	NS	0.953
Flowering Time	NS	****	*	NS	8.816 *	3.758 ****	5.573**	9.267 ***	52.121; 124.10 [$]

Table 1: Linear Mixed Model Results.

For random effects, values are variance parameters estimated using REML. Random effects were tested for significance using Likelihood Ratio Test. [@] effect was dropped from model when not significant. [$] Residual variance varied between environments (greenhouse and nursery respectively) for flowering time. NS = not significant; ASD = Anther Stigma Distance; * $p < 0.05$; ** $p< 0.01$; *** $p<0.001$; ****$p<0.0001$.

Trait	*Mean*	*σ*	$h^2_{(half)}$	*se*	$h^2_{(full)}$	*se*	H^2	*se*	*z-score*	*P*	CV_A
Anther Exertion	2.892	0.88	0.246	0.122	0.123	0.061	0.061	0.030	2.02	0.0192	15.14191
ASD	1.345	3.54	0.622	0.148	0.311	0.074	0.155	0.037	4.21	<0.0001	205.721
Corolla Length	43.469	4.59	0.617	0.149	0.309	0.074	0.154	0.037	4.14	<0.0001	8.131248
Corolla Width	1.648	0.31	0.001	0.091	4.34E-04	0.046	2.17E-04	0.023	0.01	0.5	0.556138
Petal Length	11.134	1.43	0.273	0.115	0.136	0.058	0.068	0.029	2.37	0.0089	6.646309
Petal Width	6.25	1.21	0.760	0.146	0.380	0.073	0.190	0.037	5.19	<0.0001	16.52266
Reflex angle	0.507	0.23	0.304	0.126	0.152	0.063	0.076	0.032	2.40	0.0082	23.57641
Stigma Length	47.706	4.81	0.622	0.146	0.311	0.073	0.155	0.037	4.25	<0.0001	7.823189
Sepal Length	8.068	1.06	0.162	0.111	0.081	0.056	0.040	0.028	1.45	0.0708	4.964671
Flowering Time (gh)	18.512	1.7671	0.443	0.179	0.222	0.090	0.111	0.045	2.47	0.0068	32.07856
Flowering Time (nur)	21.581	1.5607	0.233	0.094	0.116	0.047	0.058	0.024	2.47	0.0068	27.51672

Table 2: Heritability and Coefficient of Genetic Variation.

Heritabilities were calculated using untransformed data in order to calculate CV_A. Standard Error of heritability was calculated using the delta method (ref). z-scores and P values are for heritabilities. Values for flowering time were calculated separately for each environment (gh = greenhouse; nur = nursery). ASD = anther stigma distance; NS = Non-significant. SE = Standard Error. $h^2_{(half)}$ = narrow-sense heritability calculated for half-sibling families; $h^2_{(full)}$ = narrow-sense heritability calculated for full-sibling families; H^2 = broad sense heritability. CV_A = Coefficient of Genetic variation. σ = Standard deviation.

	Anther Exertion	*ASD*	*Corolla Length*	*Corolla Width*	*Petal Length*	*Petal Width*	*Reflex Angle*	*Sepal Length*	*Stigma Length*	*Flowering Time*
Anther Exertion	-	-0.2398****	0.1456****	0.0676	0.1809****	0.1377***	-0.0141	-0.0488	0.1451****	-0.0892*
ASD	-0.2701 **	-	-0.3354****	-0.0653	-0.0728	-0.0582	0.1168**	0.0188	0.3731****	0.0223
Corolla Length	0.0828	-0.3097***	-	0.05	0.492****	0.3601****	0.0796	0.232****	0.7329****	-0.0707
Corolla Width	0.0986	0.0011	0.0865	-	0.0705	0.1189**	-0.0635	-0.049	0.0119	0.0322
Petal Length	0.2868 **	-0.0724	0.2963**	0.0485	-	0.4242****	0.0544	0.1857****	0.4483****	-0.0128
Petal Width	0.1543	-0.0839	0.2192*	0.2417*	0.1583	-	-0.2295****	0.1498****	0.3257****	0.0215
Reflex Angle	0.1091	0.0554	0.0493	-0.1108	0.0817	-0.5351***	-	0.1216**	0.1612****	-0.0753
Sepal Length	-0.0182	0.0723	0.2699**	0.1158	0.2021*	0.1961*	-0.0391	-	0.2263****	-0.0496
Stigma Length	0.0617	0.4083***	0.7153***	0.0866	0.2837**	0.1884	0.1098	0.3051***	-	-0.0674
Flowering Time	-0.0317	-0.0991	0.0188	0.0829	0.1378	-0.0041	-0.0891	-0.0409	-0.0757	-

Table 3: Phenotypic and Genetic correlations between morphological traits and phenology.

Phenotypic correlations are above the diagonal and genetic correlations are below. Phenotypic correlations were calculated using the entire data set, and genetic correlations use EBLUPs of maternal families from the Mixed Models. P values were adjusted using FDR (Benjamini and Yekutieli, 2001). * = P<0.05, ** = P<0.01, *** = P<0.001, **** = P<0.0001.

POPULATION	LATITUDE	LONGITUDE
ANIMAS	31.943400	-108.877469
36	31.824067	-107.987767
37/38	32.063333	-109.180283
SOUTH FOOTHILLS	31.953163	-109.140223
NOLAND	32.076472	-109.178667

Table s1: Population collection locations.

Latitude and longitude coordinates of collection sites for this study.

FIGURES

Figure 1: Collection Locations.

Five seed collection sites used in this study from across Arizona and New Mexico. Longitude and latitude are given in Table s1.

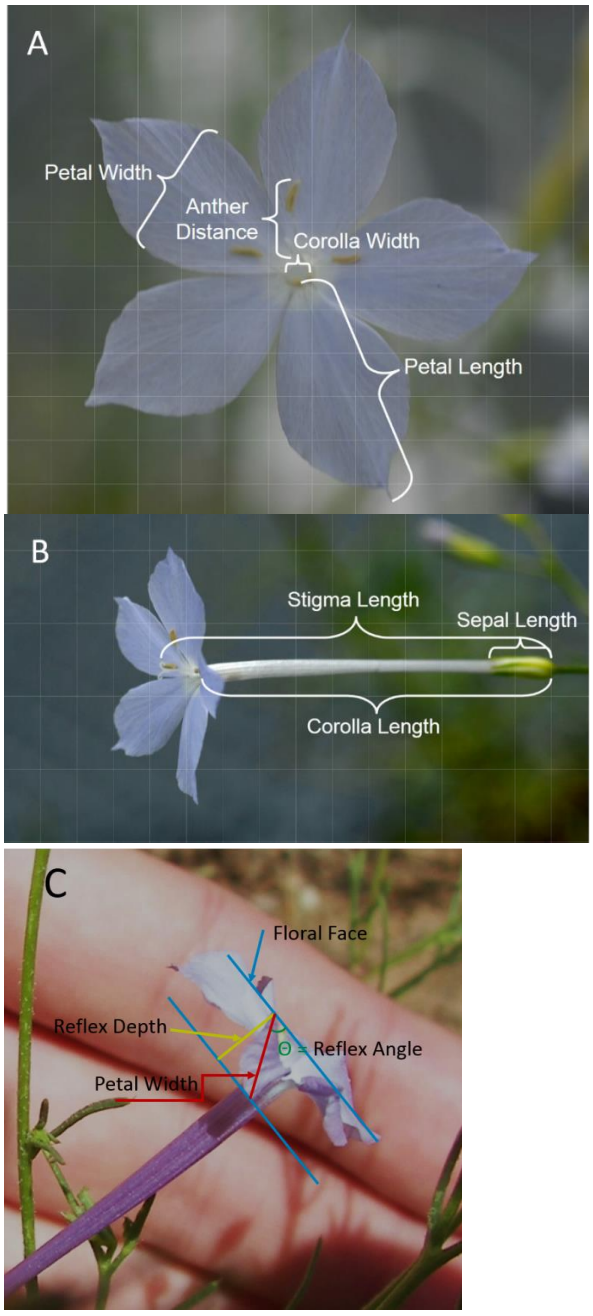

Figure 2: Floral Measurements.

A. *Ipomopsis longiflora* floral face showing petal, anther, and corolla tube opening measurements. B. Side view of *I. longiflora* flower showing floral length measurements. C. Alternate side view of *I. longiflora flower* with depiction of petal reflex measurements. Reflex depth was measured from the side using calipers and used to calculate reflex angle along with petal width (see methods).

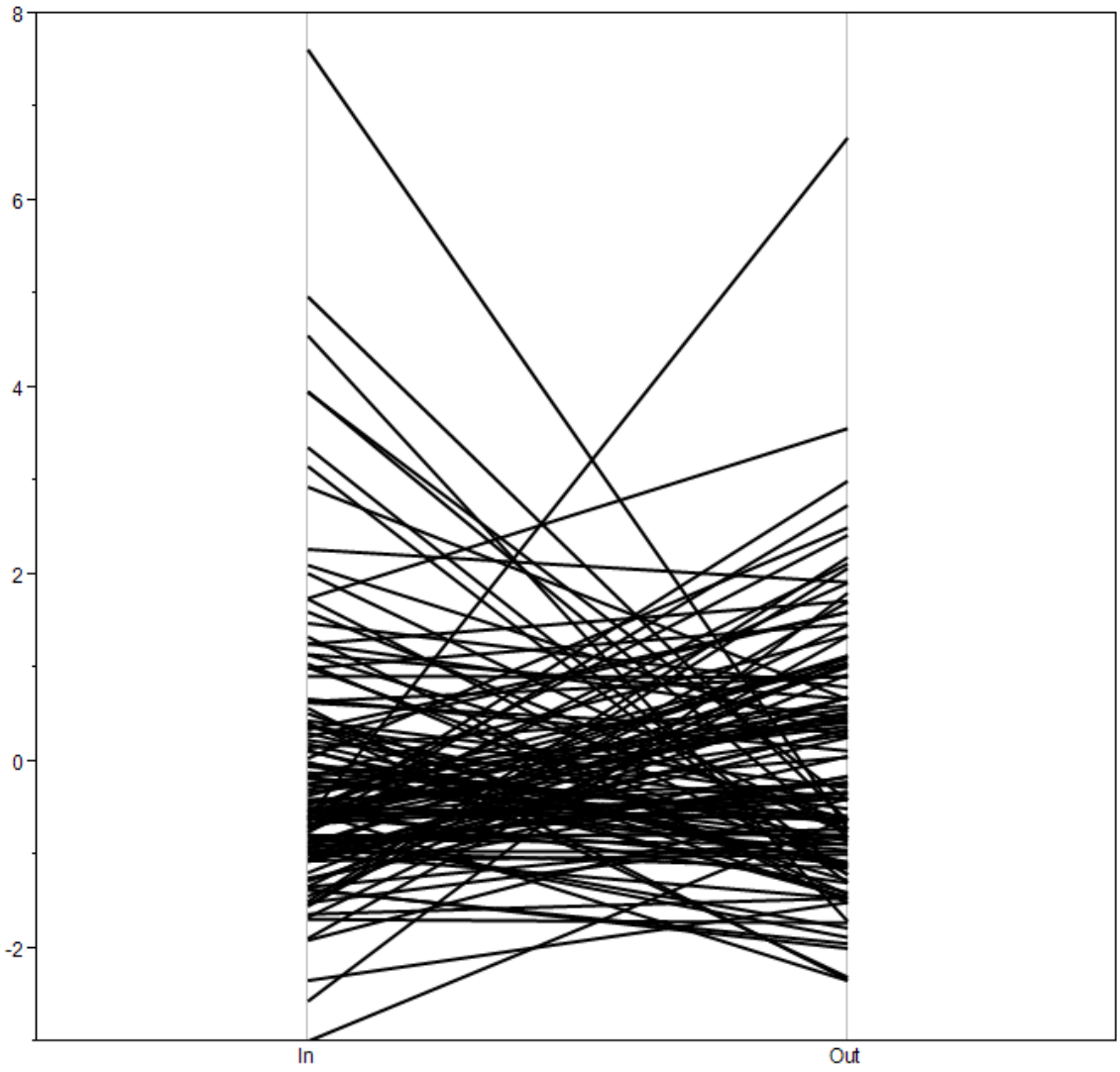

Figure 3: Genotype by Environment Interaction for Flowering Time.

Reaction norms of eBLUPs for Family x Environment. eBLUPs were calculated for Family in each Environment, greenhouse (In) and nursery (Out), using Mixed Model ANOVA.

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