ALLOMETRIC SCALING AND FLORAL SIZE VARIATION IN COLLINSIA

by

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Allometric scaling theory has previously been used to estimate the functional relationship between two biological variables. In addition to parameter estimation, deviations from the general scaling relationship can be used to create hypotheses. Here, I explore deviations from the allometric scaling pattern for plant and floral size within the genus *Collinsia* on three levels: among species, within species, and among populations of a single species. *Collinsia* species are self-compatible annual herbaceous plants that have been shown to vary in floral size, autonomous fruit production, and estimated mating system. I quantified the amount of variation in characteristics related to plant mating systems: floral size and autonomous fruit production in a pollinator-free environment and used variation and scaling deviations to generate expectations about environmental selection pressures. I found that the scaling relationships differed on each of the three levels and that deviation from the general floral size-plant size relationship is common within this genus. The among-species regression explained only 20% of the variation in floral size, and species- and population-level regressions explained even less. The four species for which I obtained controlled environment estimates of vegetative and floral trait in this study differed significantly in autonomous fruit production, floral size, and plant size, while populations of *C. heterophylla* differed in floral and plant characteristics, but not autonomous fruit

production. In addition, variation in plant size characteristics was 50-66% greater than variation in floral size characteristics suggesting selection to reduce variation in floral size and flexibility in plant size. Autonomous fruit production was correlated with floral size in *C. tinctoria*, with floral number in *C. verna*, and uncorrelated in *C. heterophylla* suggesting that floral trait and autonomous selfing ability varies among species. Using a comparative method and investigating factors correlated with plant mating system, such as floral traits, across a group of closely related species provides new insights into factors affecting their variation.

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Acknowledgements

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1. ALLOMETRIC SCALING AND FLORAL SIZE VARIATION IN COLLINSIA

Introduction

Mating systems traits effect populations via their impact on genetic variation, reproductive success, and the ability of a population to adapt (Holsinger 2000). One important factor influencing plant mating system evolution is the type of fertilization, either self-fertilization or outcross-fertilization, that results in seed production. Purely genetic models of mating system evolution weigh the benefits and costs of self-fertilization and predict that populations should evolve towards one of two evolutionary stable strategies: complete selfing (t=0) or complete outcrossing (t=1) (Fisher 1941; Lande and Schemske 1985; Lloyd 1979,1992; reviewed in Jarne and Charlesworth1993, Uyenoyama et al 1993). The benefits of selfing include reproductive assurance (Baker 1955; Jain 1976; Kalisz, et al 2004), purging of the genetic load (Husband and Schemske 1996; reviewed in Byers and Waller 1999), a two-fold transmission advantage (Fisher 1941; Jain 1976), and reduced floral display costs (Ashman and Schoen 1997). In contrast, the costs of selfing include inbreeding depression (Charlesworth and Charlesworth 1987; Uyenoyama et al 1993; Carr and

Dudash 2003), loss of genetic diversity (Charlesworth and Charlesworth 1995), pollen discounting (e.g. Holsinger et al 1984; Harder and Wilson 1998; Barrett 2003), seed discounting (Herlihy and Eckert 2002), and the potential evolutionary dead end of selfing lineages (reviewed in Takabayashi and Morrell 2001). In addition, genetic explanations of intermediate outcrossing rates propose that populations with intermediate (t) are in transition from one end of the mating system continuum to the other (Holsinger et al 1984; Lande and Schemske 1985; Schemske and Lande 1985). Clearly genetic factors and fitness benefits and costs, can play an important role in mating system evolution. The genetic models have been supported by data on wind-pollinated species (summarized in Vogler and Kalisz 2001).

In contrast, recent data from animal-pollinated populations show a wide range of outcrossing rates with 49% of the estimates between t=0.2 and t=0.8 (N= 169 studies; Vogler and Kalisz 2001). Recent theoretical models that incorporate ecological as well as genetic factors show that under certain conditions selfing, outcrossing, and intermediate outcrossing mating systems can be evolutionary stable endpoints (Holsinger 1986, 1988, 1991, 1992, 1996; Uyenoyama 1986; Uyenoyama and Waller 1991; Schoen and Brown1991; Sakai 1995; Yahara 1992; Johnston 1998; but see Sakai and Ishii 1999). Other models have found stable mixed mating when considering genetic factors and migration and/or population density (Holsinger 1986, 1991; Cheptou and Dieckmann 2002) or genetic factors and life history characteristics, such as time of first reproduction (Tsitrone et al 2003). These results suggest that plant mating systems are variable and may readily respond to selection on mating system traits (Holsinger 1991).

The production of self vs. outcross seeds may be influenced by factors such as population composition and structure. For example, a population may be comprised of a mixture of purely selfing and purely outcrossing individuals, which when averaged yield a population-level intermediate outcrossing rate. Conversely, individuals within a population can produce both outcrossed and selfed progeny. In this case, individuals may vary in the mechanism by which they self pollinate. These factors influencing mating system can vary among genera, species, populations, and/or individuals.

The production of both self and outcross progeny by an individual can enable mating system expression to be context-dependent. The fitness gain is obvious when the pollinator environment is variable and autonomous selfing occurs after the opportunity for outcross pollen receipt has past (Lloyd 1992). For example, when the pollinator environment is constant and pollinators are abundant, individuals can maximize outcross seed set, and autonomously self fertilize any remaining ovules. When pollinators are absent, the same individuals can self all ovules within a flower, and retain high relative fitness, provided there is low inbreeding depression. Such a flexible phenotype provides higher fitness to individuals than does a fixed phenotype if pollinator conditions vary and when inbreeding costs are balanced by the increased number of individuals contributed to the next generation (Schoen and Brown 1991).

Clearly, floral attractive traits and autonomous selfing ability will interact to influence the average outcrossing rate of an individual, population or species. Floral characteristics such as floral size, shape, scent, and color have been demonstrated to vary both within (e.g. Cresswell and Galen 1991; Knudsen 1994; Galen 1999; Elle and Hare 2002; Sanchez-Lafuente 2002; Frey 2004; J. Herrera 2004, 2005) and among

species (Moody and Hufford 2000; J. Herrera 2001; Armbruster et al 2002). Further, these traits directly influence pollinator attraction (e.g. Galen 1989, 1996, 1999; Conner and Rush 1996; Schemske and Bradshaw 1999; CM Herrera et al 2001; Sanchez-Lafuente 2002; Elle and Carney 2003). Studies of the relationships between flower morphology, pollinator attraction, and outcrossing rate (Holtsford and Ellstrand 1992; Fausto et al 2001; Elle and Hare 2002; Elle 2004) indicate that species with small flower size are typically more highly selfing while those with larger flowers are typically more outcrossing. Pollinator attraction traits are costly (Galen 1999, 2000; Ashman and Schoen 1997; Andersson 2005). Therefore, if a species reproduces primarily through self-pollination, the production of expensive secondary attractive traits like large flowers, high nectar volume and quality, and scent are disfavored. Thus, floral attractive traits are often used as a surrogate of mating system (reviewed in Takebayashi and Morrell 2001).

One powerful approach for exploring variation in flower size is to consider the allometric relationship between plant size and flower size. Allometric scaling typically quantifies the change in the relative dimensions of one aspect of morphology as a function of changes in another (Niklas 1994; Gayon 2000) by regressing two variables of interest and determining their functional relationship (Niklas 1994, 2004; see Ushimaru and Nakata 2001, 2002). Allometry has been applied to a wide range of topics including biomass allocation, species packing, or large scale patterns such as the scaling relationship between metabolic rate and body size (see West, Brown and Enquist 1997, 1999, 2000). The focus of these analyses is a precise determination of allometric scaling coefficients as an estimator of the functional relationship between a

variable of interest and body size (Niklas 1994, 2004). This is the typical application of allometric analysis. In contrast, a less-used application of allometric analysis focuses on the distribution of data points around the allometric scaling line to generate hypotheses about factors that may be influencing deviations (Niklas 1994). For example, large deviations from the general scaling relationship suggest that those taxa significantly differ in the environment they experience. In the case of floral size vs. vegetative size, variation around the allometric scaling line can suggest relative changes in flower size/body size due to differences in environmental selection pressures. For plants in general, vegetative features are expected to indirectly affect fitness and are likely to be more plastic, while features directly tied to reproduction, such as floral traits, may be under strong selection to reduce variation (Niklas 1994; Conner and Sterling 1995; Sherry and Lord 1996; Armbruster et al 1999). While the pollinator environment may directly select on floral size, developmental and genetic factors shared by group of closely related species can constrain the response to selection (Arthur 2003).

In the genus *Collinsia* (Plantaginaceae), the 18+ species produce a wide range of floral sizes. All species in this genus are self-compatible and differ in the timing of autonomous selfing (Armbruster et al 2002). Several of the smaller flowered species autonomously self-pollinate early in a flower's lifespan, while the large-flowered species self-pollinate late in a flower's lifespan (Armbruster et al 2002). Variation in the timing of selfing (Armbruster et al 2002), floral morphology, and development (Kalisz et al 1999) within this genus could contribute to the evolution of diverse floral sizes observed in *Collinsia*. Here I quantify the allometric scaling relationship between flower size and plant size using both published data and controlled-environment experimental data to

ask three levels of questions. First, I use published data for the genus *Collinsia* to ask: What is the scaling relationship for this genus? Do individual species show strong deviations from the scaling line? Second, I use the experimental data for four species, to ask: Does the scaling within a species differ from that for among species? Finally I use individual allometric analyses for three populations within a single species to ask: Do populations differ from each other in their scaling relationships? Further, I explore phenotypic differences among species and the underlying phenotypic correlations among floral traits and vegetative traits within species.

Methods

The study system: *Collinsia* (18+ species) and *Tonella* (2 species) constitute tribe *Collinsieae*. Fifteen species are found exclusively in western North America. *Collinsia parviflora* is found throughout the west, north to Alaska and east to Ontario, Canada, while *C. verna* and *C. violacea* are currently restricted to the eastern half of the United States. All *Collinsia* species are self-compatible winter or spring annuals, which germinate and grow in the winter or early spring and bloom in the early spring to early summer. Flowers in Collinsieae are zygomorphic, with a 5-lobed calyx, a 2-lipped corolla with a constricted tube, four stamens and one pistil, containing 2 to many ovules. The corollas of *Collinsia* have one folded ventral petal that forms a keel, enveloping the pistil and stamens. All species secrete nectar and even the small flowered species have been observed being visited by pollinators (W.S. Armbruster, pers obs). A variety of

solitary bees, including *Osmia* spp. (Megachilidae), *Anthophora* spp., and *Emphoropsis* spp. (Anthophoridae), and less frequently, *Bombus* spp., and *Apis mellifera* (Apidae) pollinate this tribe.

The species of Collinsieae studied to date show variation on a basic theme of sequential protandry. In general, the four anthers dehisce one at a time. Each staminal filament elongates just prior to anther dehiscence, placing the anther and pollen at the tip of the keel petal. The style elongates late in development (most larger-flowered species) or remains approximately the same length (many smaller-flowered species) (Armbruster et al 2002). Self-pollination can occur when the stigma contacts anthers or free pollen in the front of the keel. Dramatic variation in the timing of stigma-anther contact exists within and among species (Armbruster et al 2002) and ranges from prior to delayed selfing (sensu Lloyd 1992). Flowers last 2-7 days in most *Collinsia* species. Species range from primarily selfing to primarily outcrossing (Garber 1975; Mayer et al 1996; Armbruster et al. 2002; Kalisz et al 2004). Variation in floral size and floral morphology was examined in a phylogenetic context by Armbruster et al (2002), revealing pairs of sister taxa throughout the phylogeny where one species has small flowers and the other species has large flowers (Figure 1).

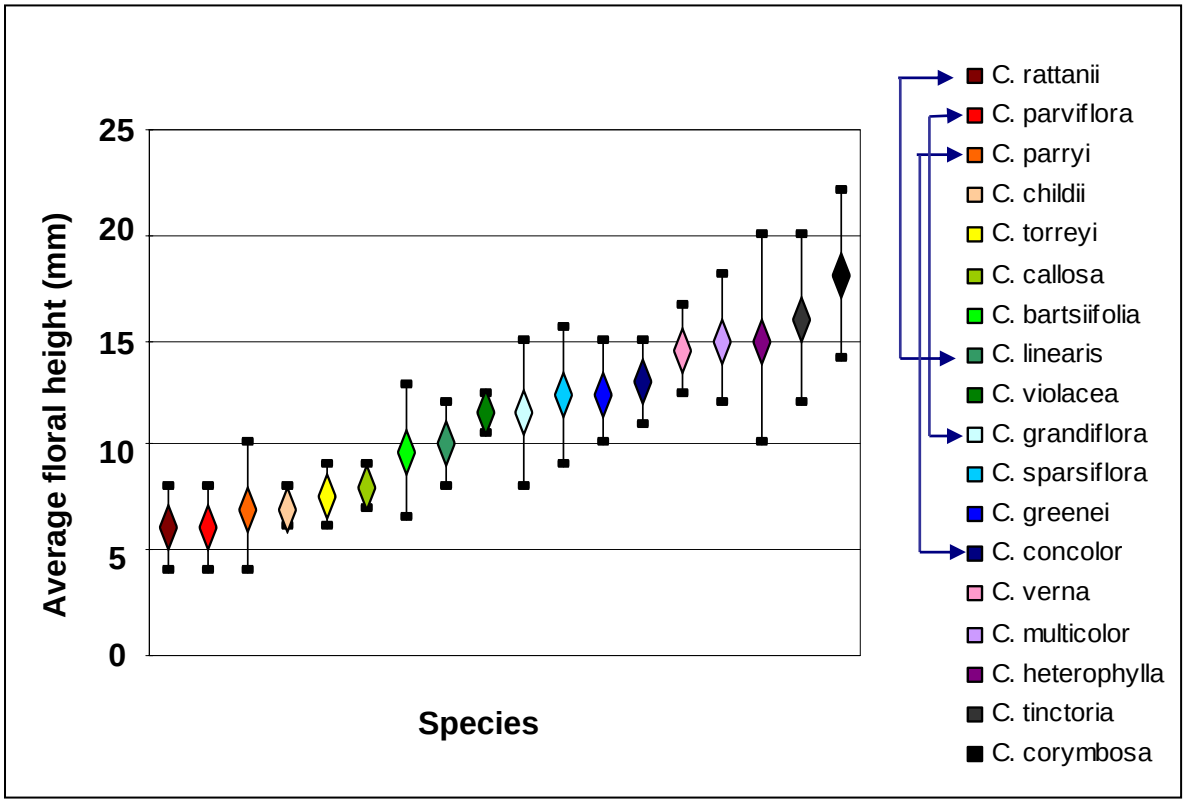

Figure 1: Variation in Corolla Size with diamonds representing average corolla height and error bars representing the range of height as specified in the Jepson's Manual (Neese 1993) and Gray's Manual of Botany (Gray 1970). Color coding of species is consistent among Figures1-5. Small and large sister taxa are connected by arrows in the legend (data from Armbruster et al 2002).

Data used in analyses: I used data from the Jepson's Manual of Higher Plants of California (*Collinsia* (Neese 1993) in Hickman (ed.) 1993) for the western species. Contributors to the Jepson's Manual, such as Neese, are experts in the taxonomic group they describe. In producing the treatment for the genera, each contributor was required to supplement current knowledge with any existing literature and **all** available herbarium sheets for the group. Thus the data for *Collinsia* are accurate and complete. For the two eastern species, *C. violaceae, C. verna,* I used data from Gray's Manual of

Botany; Gray 1970). From both of these sources, I obtained estimates of flower size, which was estimated by average corolla height. Values of vegetative plant height were calculated as the average of the minimum and maximum size reported for each species. These data were used to generate the general scaling relationship of flower size and plant size for the genus. All other analyses (among four species and three populations within one species) were conducted on data derived from my controlled environment and greenhouse experiments, described below.

Controlled environment estimates of flower and vegetative traits: *Among-species*—To quantify variation within and among species in floral and vegetative traits and to determine if individual species differed from the general scaling relationship I used four species that were similar for flower size but varied in plant size; *C. concolor*, *C. heterophylla*, *C. tinctoria*, and *C. verna*. Bulk-collected seeds of all four species were used in the experiments: *C. verna* (GPS 41^{o} 35.32' N, 80^{o} 21.35' W), *C. tinctoria* (38^{o} 26' N, 122^{o} 58' W)*, C. concolor* (33^{o} 34' N, 119^{o} 01' W)*, and C. heterophylla* (34^{o} 27' N, 119^{o} 08' W). The seeds were placed onto wet paper towels in Petri dishes in a 4^{o} C refrigerator until they germinated. Upon germination, seeds were planted into 96 well trays in Sunshine germination mix ™ and placed in Percival growth chambers (10^{o} C day, 5^{o} C night, 10 hour days). When the plants had grown at least one set of true leaves, individuals were transplanted into 48 well trays containing Fafard #4 ™ and placed into the greenhouse (12-18° C night, 18-24^{o} C day, natural day length). When the plants grew larger, they were transplanted into 3 inch pots containing Fafard #4 and grown to senescence.

Because of space constraints in the growth chambers for germination, the plants were grown in two sequential experimental blocks. Difficulties with overheating in the greenhouse during the first block caused premature senescence of many of the flowers from the plants and the majority of the *C. concolor* plants were killed. Thus, the data from Block 1 are only included to indicate the effect of temperature variation, but are excluded from all other analyses. Because there were few seeds of *C. concolor* remaining after the first block, sample sizes were significantly smaller for this species than for other species (Table 1).

Table 1: Sample sizes *Collinsia* species and population (A-C) sample sizes for variables used to estimate autonomy rate, plant size (# of branches, vegetative display size, dry above-ground biomass, and mainstem height), and flower size (# of flowers, total dry floral weight, floral height, floral width, floral depth, and floral area).

variable	*C. heterophylla A*	*C. heterophylla B*	*C. heterophylla C*	*C. concolor*	*C. tinctoria*	*C. verna*
autonomy rate	22	33	40	6	44	50
# of branches	25	34	37	8	40	50
vegetative display size (cm)	25	34	36	8	40	50
dry above-ground plant biomass (g)	23	32	38	7	48	48
mainstem height (cm)	25	34	37	8	40	50
# of flowers	22	33	40	6	44	48
total dry flower weight (mg)	22	33	38	6	35	42
floral height (mm)	25	35	40	8	46	55
floral width (mm)	25	35	40	8	46	55
floral depth (mm)	25	35	40	8	46	55
floral area (h*w) (mm^2)	25	35	40	8	46	55

Within species-- Three populations of *C. heterophylla* were used to quantify the extent of population-level differences in allometric scaling. The GPS locations of the three populations are A (38^{o} 38' N, 121^{o} 13'W), B (34^{o} 27' N, 119^{o} 08' W), and C (37^{o} 28' N, 120^{o} 04' W). This species was previously shown to have variation in both floral morphology (Charlesworth and Mayer 1996), development (Armbruster et al 2002), and outcrossing rate (Charlesworth and Mayer 1995). The germination and growth conditions of these populations were identical to those described above.

Traits measured: For all plants grown in this experiment, the following traits were measured:

Flower size: Starting with the second flowering whorl, one fully mature flower per whorl was carefully removed from the main stem of the plant by clipping the petiole close to the stem. To minimize water loss in the flowers, only 5 flowers were collected at a time in the greenhouse and transported to the lab in a covered 36 well tray for immediate weight and size measurements. Each flower was weighed to the nearest 0.001mg on a Mettler microbalance. Next, the total height width, and depth of the each flower was measured to the nearest 0.01 mm using digital calipers (Figure 2).

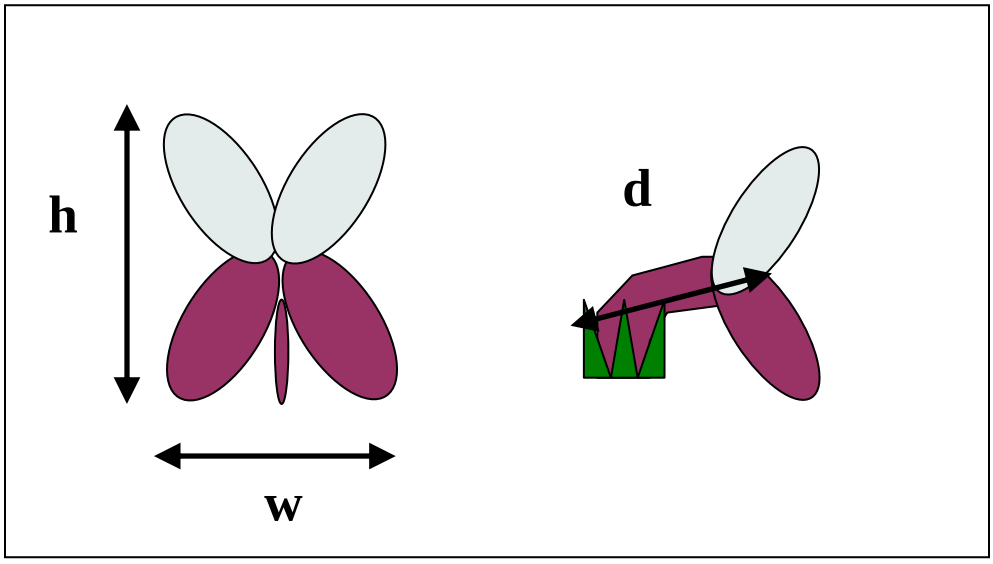

Figure 2: Illustration of Measurements used to estimate floral height (h) width (w) and depth (d). Floral area was estimated by a= h*w.

The flower was then photographed using a digital camera attached to a microscope and the image stored using the Optimus 6.5 image analysis software program. Flower number**:** Total flower number was scored as the sum of the number of flowers used for floral size measures, number of flowers that did not set fruit, and the number of fruits at the final harvest of the plants (senescence). Floral fresh/dry weight: Dry floral weight for one flower per plant (fourth whorl), and fresh weight of all flowers used in floral size estimations, were measured to the nearest 0.001 mg with fresh weight measured directly after removing the flower from the plant, and dry biomass measured after the flowers have been dried for at least 24 hours in a 40^{o} C degree drying oven. Total fresh floral weight was calculated by multiplying the average fresh floral weight of an individual flower by the total number of flowers. Total dry floral weight was estimated by multiplying the single dry floral weight taken for each individual by the total number of flowers. Autonomous fruit production- All flowers not collected for floral size measurements were unmanipulated and allowed to autonomously self in the pollinator-free greenhouse. Autonomy rate was calculated as the (total number of fruits)/ (total number of flowers) produced by each individual.

Plant size: The number of branches, length of each branch, and length of the main stem was measured for each plant. The average branch length for each plant was calculated, and an estimate of vegetative display size was determined by summing the length of the main stem and of all of the branches for each individual. Plant biomass**:** Above ground fresh plant biomass was measured at plant senescence by removing all of the flowers and fruits and weighing all vegetative material to the nearest 0.001g on a

Mettler microbalance. All plant material was then dried in a drying oven and reweighed to obtain above ground dry plant biomass estimates.

Data Analysis

Allometric scaling :

Model selection: Both power and linear equations are used in allometric analyses (Niklas 1994, 2004). When obtaining the scaling coefficient is the object of the analysis, a power function is generally used ($y=bx^k$). The scaling coefficient (k) is the slope of the regression and represents the general allometric trend from the data (Niklas 1994). Alternatively, the linear equation $y=kx + b$ can be used when the scaling coefficient is not the object of the analysis. In addition a Model I Least Squares (LS) regression considers y to be the dependent. The LS regression assumes that values of the independent variable (x) do not randomly vary, that the expected relationship between X and Y is linear, that the error term is normally distributed with a mean of zero, and the distribution of y is normal for each value of x (Niklas 1994, 2004). Often these assumptions are violated in biological systems, and Model II Reduced Major Axis (RMA) regression is used, where x and y are both considered dependent variables.

Model I power and linear analyses were run on the data sets for all levels and were found to give quantitatively identical results. Therefore all analyses presented here used Model I LS linear regression. While LS can often underestimate the scaling

variables, the scaling coefficient estimation was not the focus of this study. Future analyses will further consider the use of RMA regression instead of LS regression.

Allometric data is often log-transformed before regressing to increase normality, decrease heteroscedasticity, to increases the correlation coefficient between the variables, or to more easily examine proportionality regardless of the units of measure (Niklas 2004). In general, log-transformation is used when the two variables differ by at least two orders of magnitude. The fit of my data were not improved by log- or semi-log transformation, values of both variables differed by less than two orders of magnitude, and the correlation between the variables was not increased by log-transformation. Hence, my data were left untransformed.

In my analyses, I used the linear equation, y=kx +b, where y is flower size, x is plant body size, b is the constant origin index, and k is the constant ratio of the change in size of the studied organ to the change in body size of the organism (Niklas 1994). The scaling relationships were calculated across all species within the genus using published data, among three species (*C. heterophylla, C. tinctoria,* and *C. verna*) using controlled environment data, and among three populations of *C. heterophylla* using controlled environment data. In addition, I regressed floral **area** on plant height at the population, species, and among-species levels to determine if the variable used to represent floral size changed the scaling relationship. Scaling coefficients were compared among species, among population, and in relation to the coefficient obtained in the regression of the published data. R^2 values were obtained to determine the amount of variation in a species or a population explained by the regression.

Floral and vegetative trait variation analyses:

I ranked each of the species and subspecies for the average floral size (corolla height) and for the average plant size (plant height). Rank order of the species and subspecies were compared to determine if the order changed for the two variables (Figure 3).

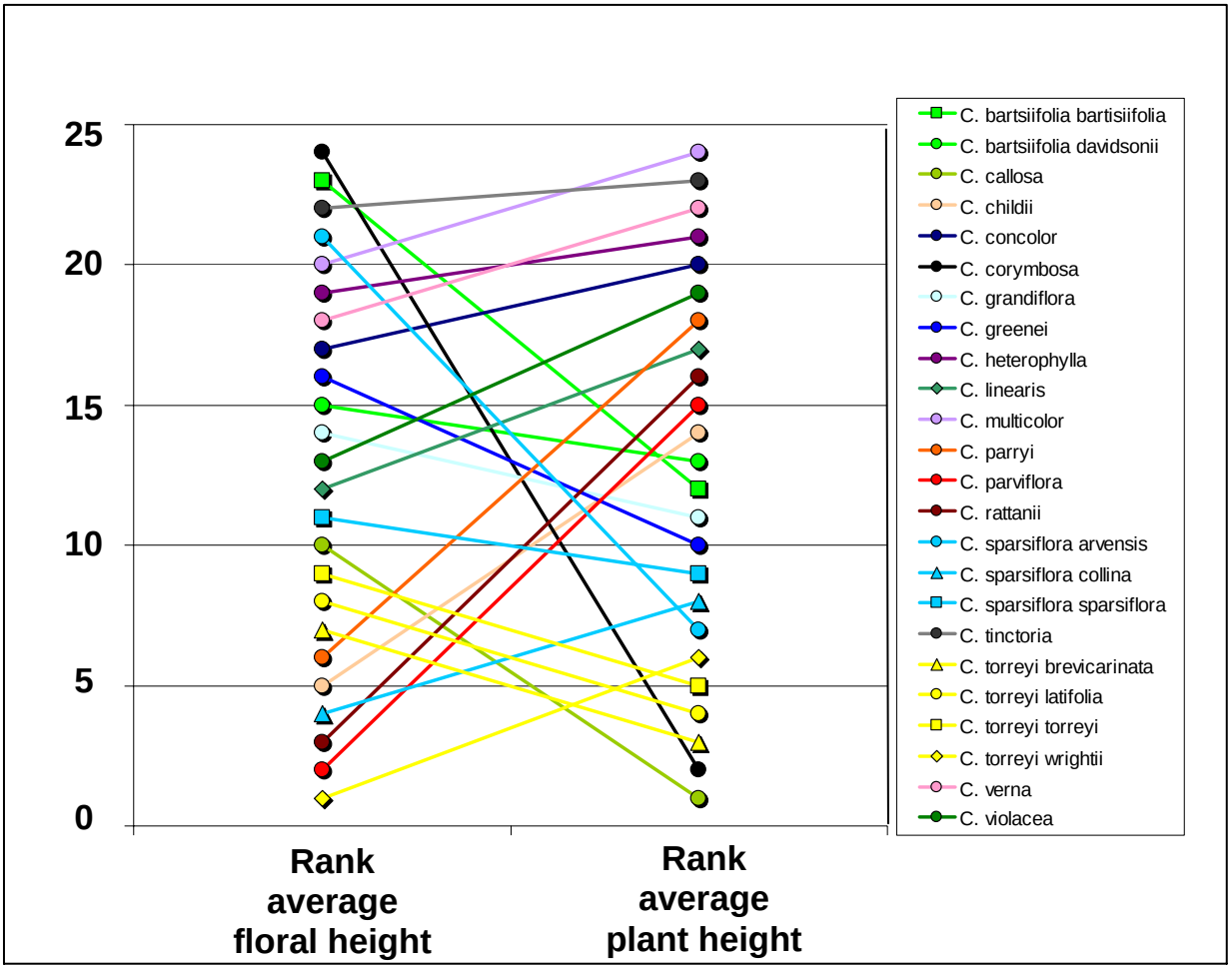

Figure 3: Rank Order Graph : comparison of floral height and plant height using the same published data from Figure 1. Species that have listed subspecies are coded with the same colored line, but differing symbols. Subspecies with equivalent average heights were ranked alphabetically for those equal in rank.

I quantified variation in floral and plant morphological traits both within and among species. I calculated the mean, variance, and standard error for floral characteristics (number of flowers, floral height, floral width, floral depth, floral area, and total dry floral weight and autonomy rate,) and plant characteristics (main stem height, display size, number of branches, and dry plant weight). Due to unequal sample sizes (Table 1), standard errors are reported for all analyses. I used univariate Analysis of Variance (Type III sums of squares) to determine if species differed for each variable (species as a fixed factor). Tukey's post-hoc test was used to determine which species or populations were significantly different. Due to the significantly smaller sample size of *C. concolor* in Block 2, I ran the ANOVAs both with and without *C. concolor*. Since there were no significant differences in my results by including *C. concolor*, the results are reported with *C. concolor* included. I determined if there were correlations among the seven floral characteristics using Pearson's Correlation and Spearman's Rank Correlations.

Since the sample size of the *C. heterophylla* population *B* was most similar to the sample sizes of the other two species, Population B was used in the among species analyses. For the within C. heterophylla analyses, Populations A, B and C were used. Unless noted, all analyses include block 2 data only.

Results

Among-species scaling in the genus, *Collinsia:* The general allometric scaling relationship between plant and flower size for the genus *Collinsia* indicates a general increase in flower size with plant size (Figure 4; Table 2). The slope of the regression is 0.22. However, the regression only explained 20% of the variation in the data.

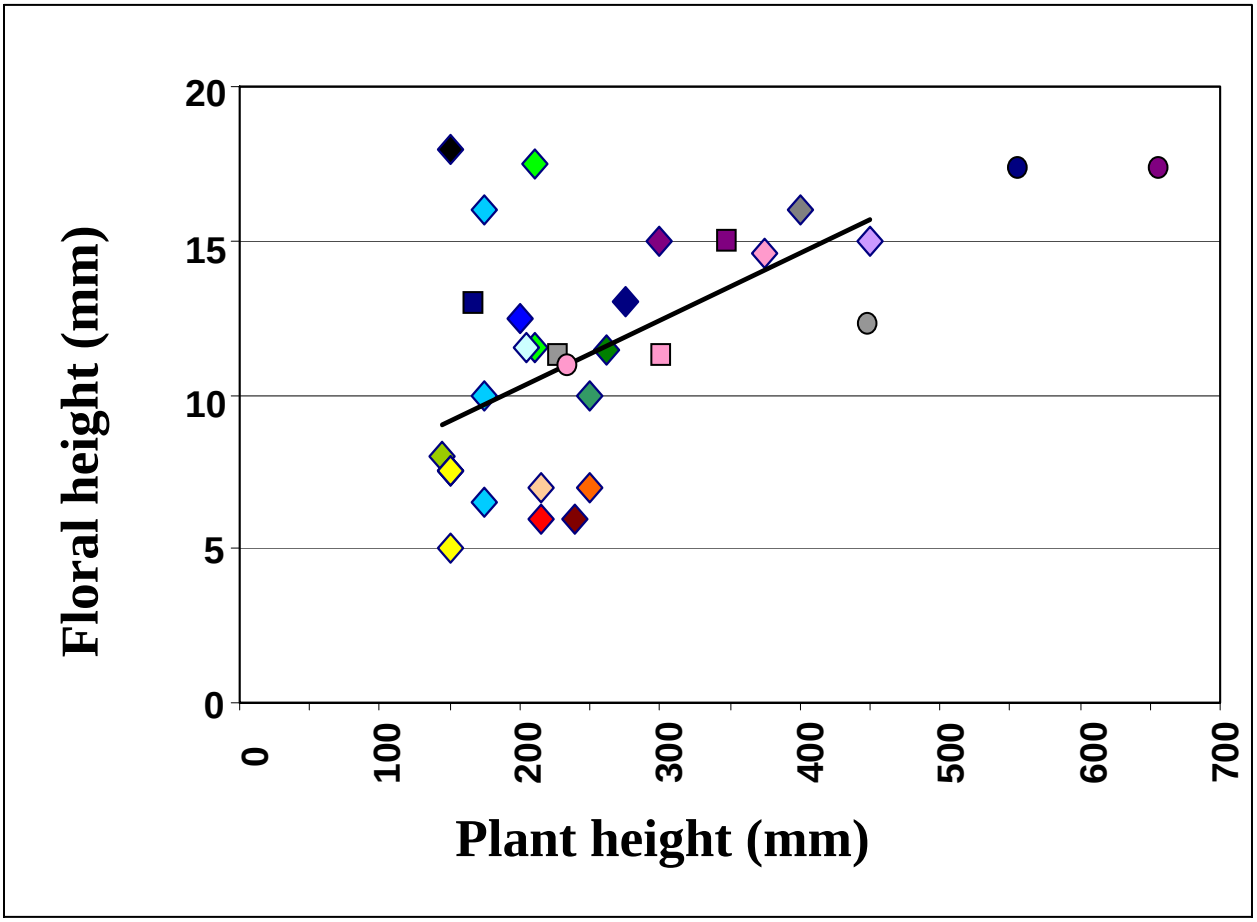

Figure 4: Allometric Regression using both the published data (diamonds) Neese 1993, Gray 1970) as well as block 1 (squares) and block 2 (circles) estimates from this study. There are three species of C. torreyi that overlap (150, 7.5) and are represented by a single yellow diamond. The regression line represents the general trend for the published data only ($y=0.22x + 5.85$ $R^2= 0.20$ $p=0.029$).

Table 2: Parameter Estimations and Confidence Intervals

			95% CI			95% CI			
floral variable	***Collinsia species***	**b**	**lower**	**Upper**	**k**	**lower**	**upper**	**r^2**	**p**
height	*heterophylla A*	17.913	15.216	20.611	-0.002	-0.007	0.003	0.036	0.365
height	*heterophylla B*	15.131	12.258	18.004	0.004	0.000	0.008	0.095	0.076
height	*heterophylla C*	15.351	12.829	17.874	0.003	-0.001	0.007	0.067	0.126
height	*tinctoria*	9.273	7.198	11.348	0.006	0.002	0.010	0.176	0.009
height	*verna*	10.358	9.213	11.503	0.002	-0.003	0.006	0.013	0.441
area	*heterophylla A*	247.880	184.010	311.750	-0.052	-0.161	0.056	0.042	0.328
area	*heterophylla B*	192.669	130.940	254.398	0.098	0.008	0.189	0.133	0.034
area	*heterophylla C*	236.061	182.698	289.425	0.031	-0.056	0.118	0.015	0.474
area	*tinctoria*	35.184	-7.241	77.610	0.205	0.116	0.293	0.379	0.000
area	*verna*	93.603	77.052	110.153	0.036	-0.029	0.101	0.027	0.269

I then plotted the value of each species and population grown in the greenhouse in Blocks 1 and 2 on to the general allometric regression graph (Figure 4). For all species, the vegetative heights of Block 1 plants (Figure 4 squares) are lower than the published values (diamonds), while Block 2 plants (Figure 4 circles) are shifted toward larger plant height values. In contrast with plant height, floral height does not change dramatically between the experimental blocks. For all four species in my experiment, the coefficients of variation (CV) are 50 to 66% greater for plant height than for flower size (Figure 5).

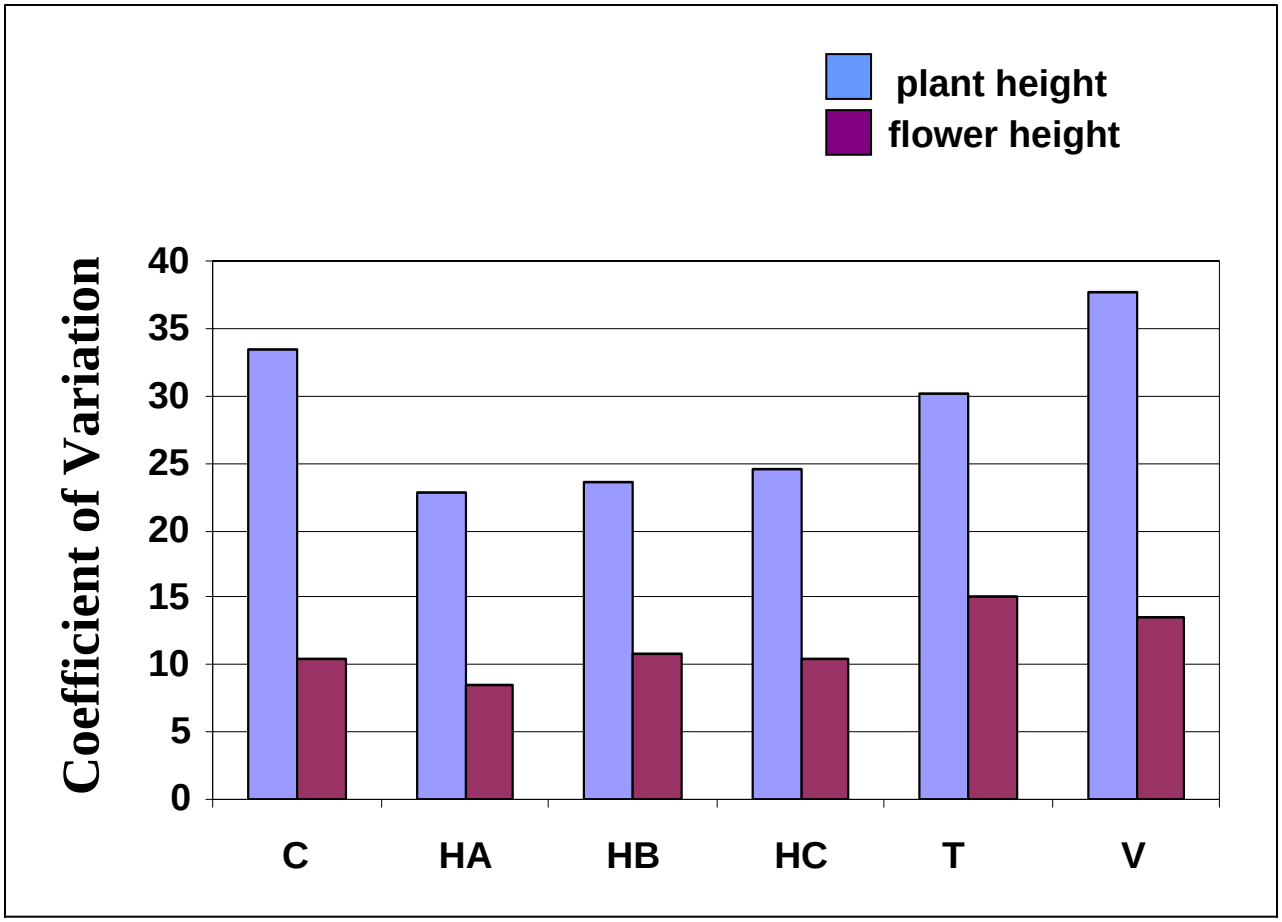

Figure 5: **Coefficients of Variation** for plant height and flower height based on experimental estimates from block 2 only. C= *C. concolor*, HA= *C. heterophylla A*, HB= *C. heterophylla B,* HC= *C. heterophylla C*, T= *C. tinctoria*, V= *C. verna.*

The separate allometric regression for each species revealed that the slope and the intercept of the scaling relationship for *C. heterophylla*, *C. tinctoria*, and *C. verna* differed from the overall allometric line calculated for the genus (Compare Figure 4 to Figure 6a, b; Table 2).

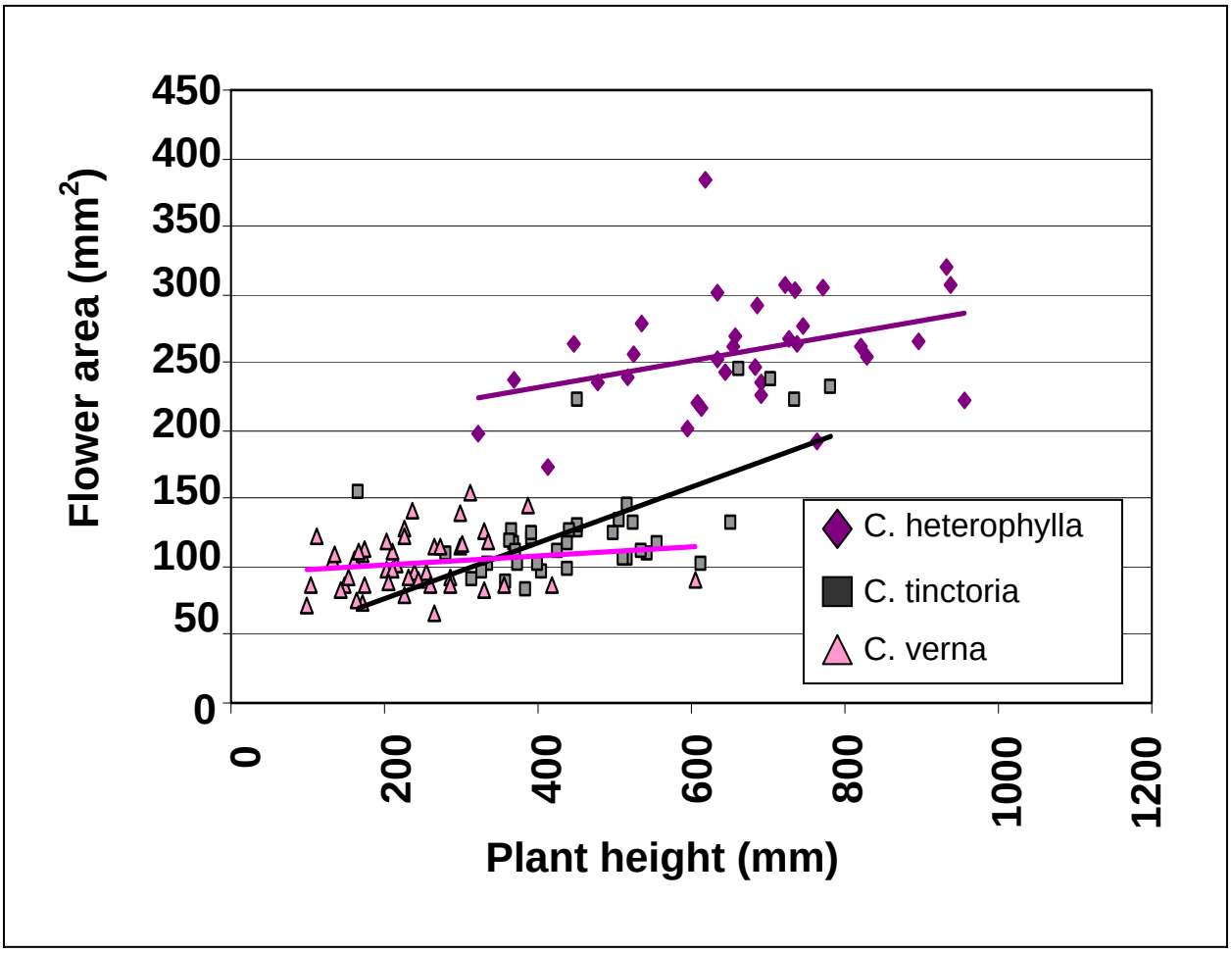

Figure 6a: **Among Species Allometric Height Regression** : Scaling relationships among 3 species of *Collinsia* using floral area as the estimate of flower size and plant height as the estimate of plant size. *Collinsia heterophylla* (population B) (y=0.098x + 192.67, R^2= 0.13 p=0.034), *C. tinctoria* (y=0.205x + 35.18, R^2= 0.38 p=0.000), and *C. verna* (y=0.036x + 93.60, R^2= 0.03 p=0.269).

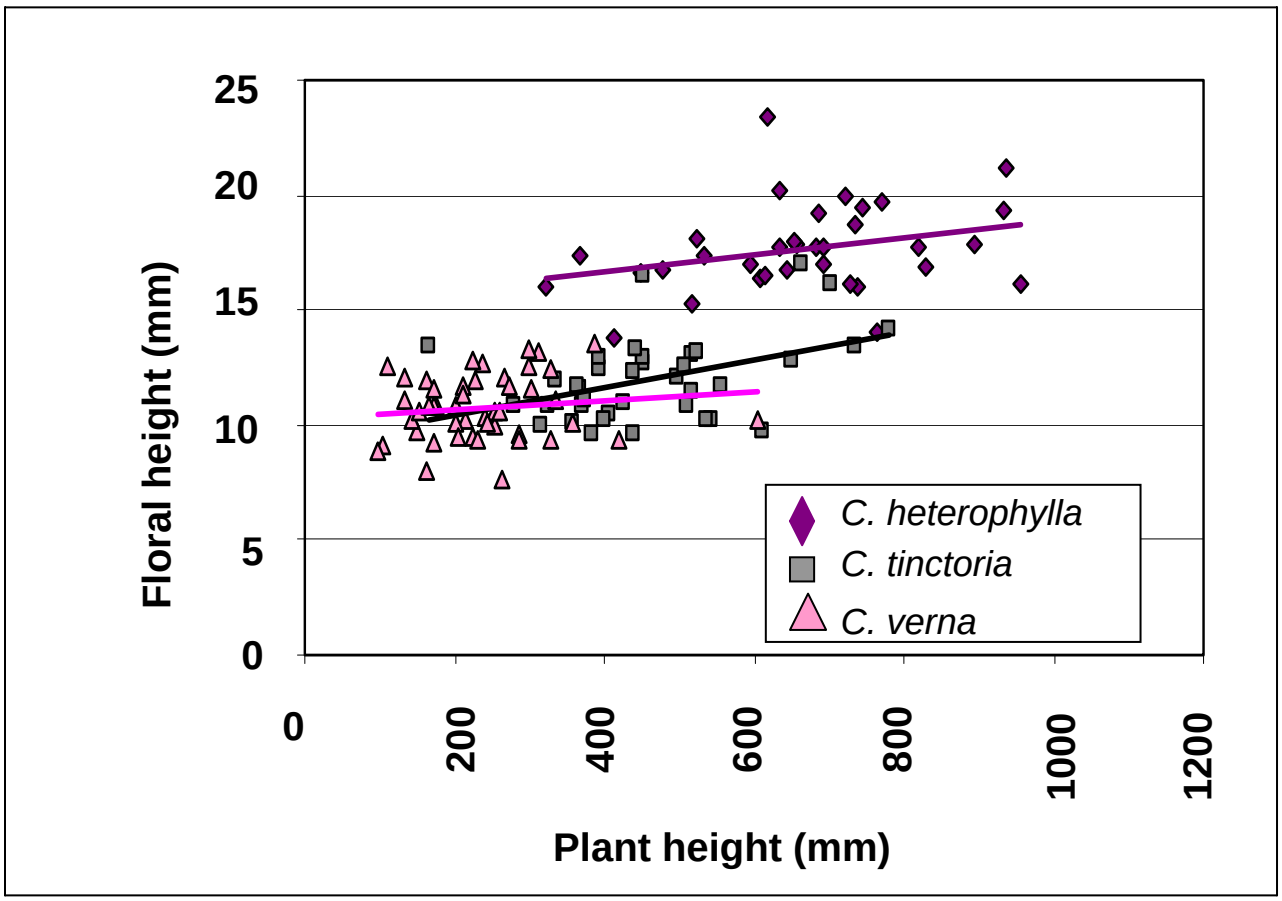

Figure 6b: **Among Species Allometric Area Regression** : scaling relationships among 3 species of *Collinsia* using floral height as the estimate of flower size and plant height as the estimate of plant size. *Collinsia heterophylla* (population B) (y= 0.004x + 15.13, R^2= 0.10 p=0.076), *C. tinctoria* (y=0.0059x + 9.27, R^2= 0.18 p=0.009), and *C. verna* (y=0.0017x + 10.36, R^2= 0.01 p=0.441).

The scaling coefficient (k) is not constant among species, or among populations of *C. heterophylla*. In addition, the scaling coefficients of all regression calculated from the greenhouse grown-plants were different from the scaling coefficient calculated using the published *Collinsia* data. The plot of floral area versus plant height revealed that *C. verna* is highly variable in both plant and floral height and floral size could not be explained by allometric scaling. *C. tinctoria* showed smaller deviations from the scaling relationship when compared to *C. verna*. When I used floral area versus plant height, I found that the regression for *C. verna* was again not significant, and the regression for *C. heterophylla* became marginally significant.

Population-level scaling relationships: All three populations of *C. heterophylla* had scaling relationships that differed from the overall scaling line (Figure 7a, b Table 2). Only Population B of *C. heterophylla* had a significant regression, with the allometric regression explaining 13% of the variation in floral area and 10% of the variation in floral height. The regressions in *C. heterophylla* populations A and C were not significant for either floral area or floral height.

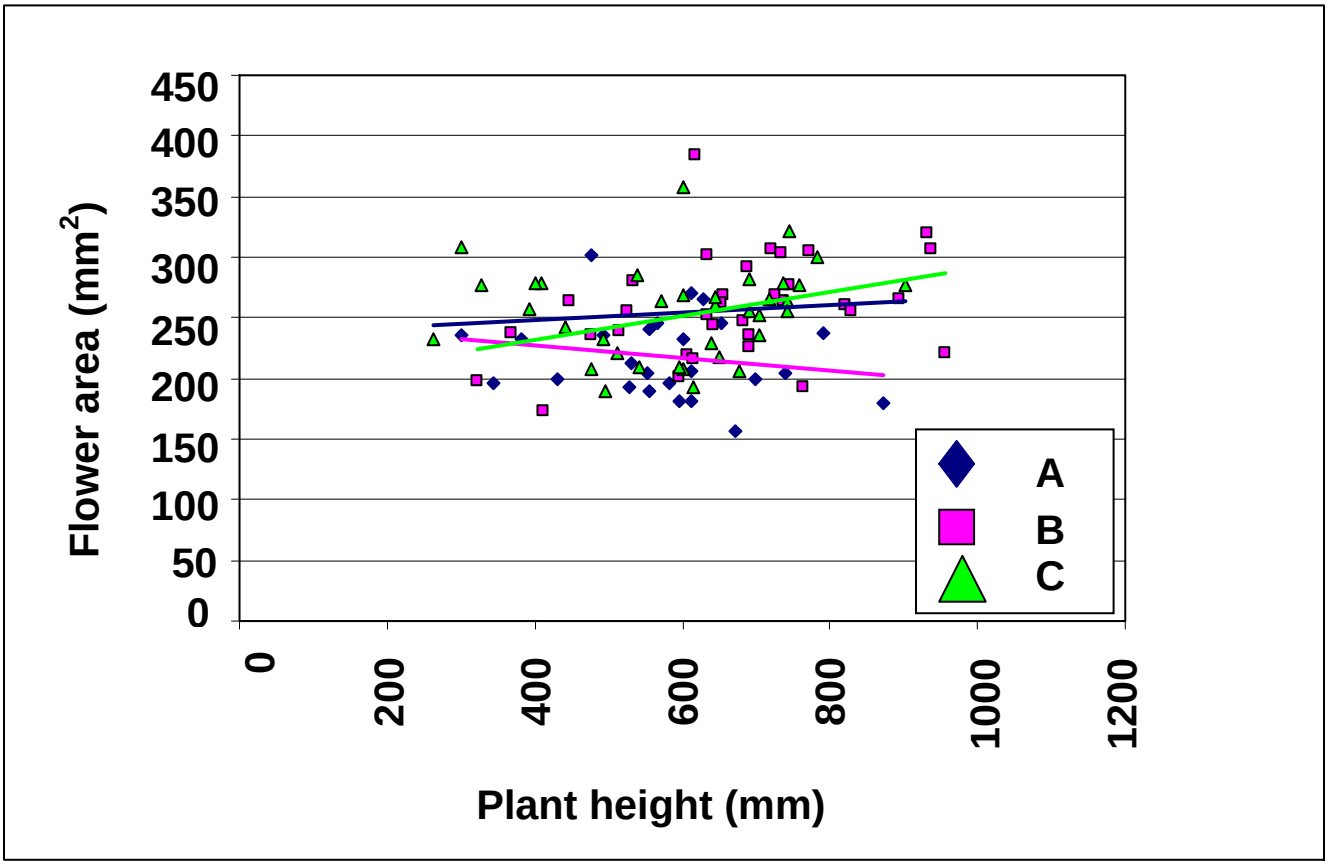

Figure 7a: **Among Population Allometric Area Regression** : Comparison of allometric scaling relationships among 3 populations of *C. heterophylla* using floral area as the estimate of flower size and plant height as the estimate of plant size. *Collinsia heterophylla A* (y=-0.052x +247.88, R^2= 0.04 p=0.33), *C. heterophylla B* (y=0.098x +192.67, R^2= 0.13 p=0.034), and *C. heterophylla C* (y=0.031x + 236.06, R^2= 0.02 p=0.47).

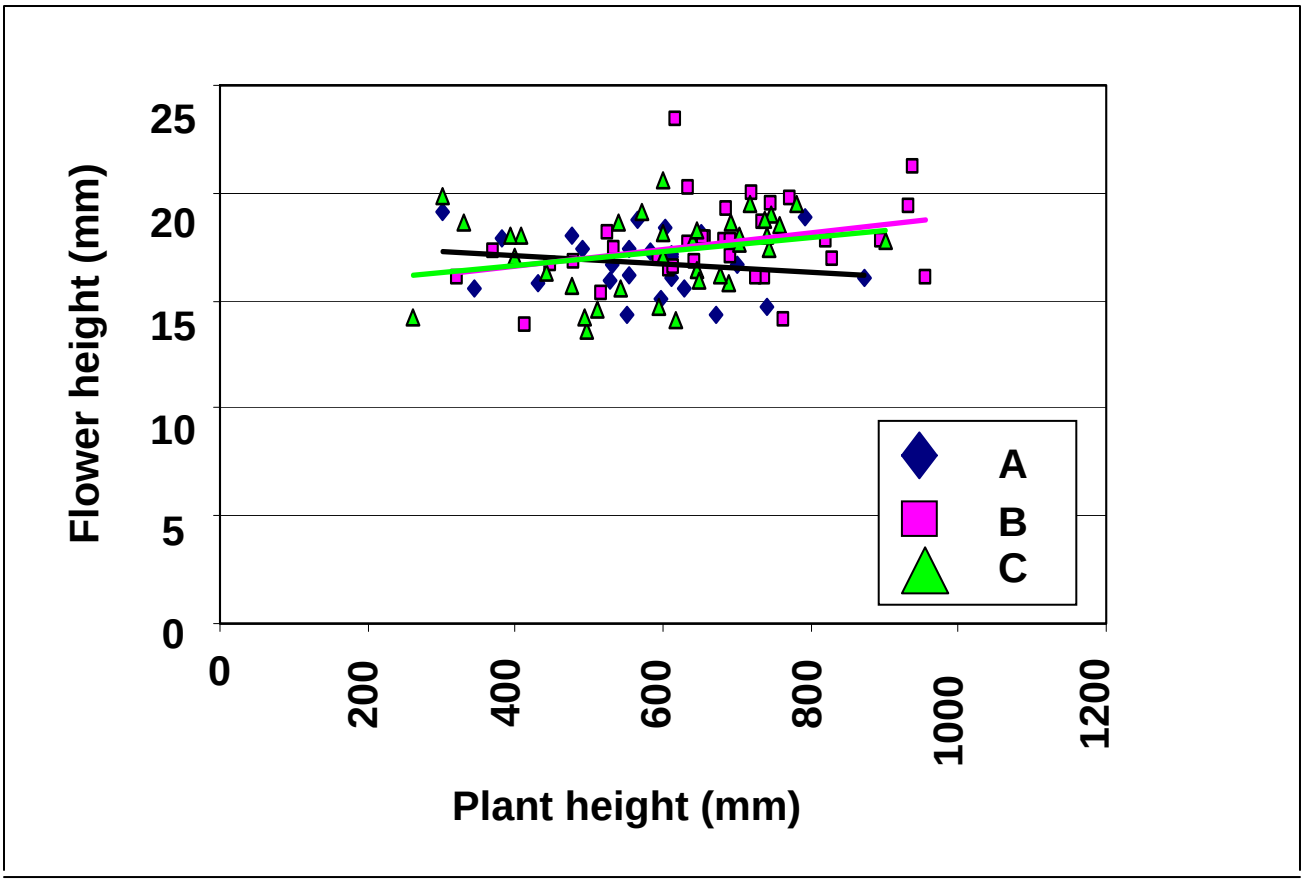

Figure 7b: Comparison of allometric scaling relationships among 3 populations of *C. heterophylla* using floral height as the estimate of flower size and plant height as the estimate of plant size. *Collinsia heterophylla A* ($y=-0.002x + 17.91$, $R^2= 0.04$ $p=0.365$), *C. heterophylla B* ($y=0.004x + 15.13$, $R^2= 0.10$ $p=0.076$), and *C. heterophylla C* ($y=0.003x + 15.35$, $R^2= 0.07$ $p=0.126$).

Among species variation in floral traits: The species of *Collinsia* used in this analysis vary in average corolla height from ~ 6 mm to 16 mm, with ranges that significantly overlap (Figure 1). When the species and subspecies were ranked in order of flower size and plant size, I found that the rank order for floral size was significantly different from the order of the species for plant size (Figure 3).

The species differed significantly in floral characteristics and plant size characteristics (Table 3).

Table 3: ANOVA Results Among Species

variable	type III sum of squares	degrees of freedom	mean square	F	p-value
autonomy rate	0.21	3	0.07	7.25	0.000
# of branches	895.08	3	298.36	19.94	0.000
vegetative display size (cm)	262306.18	3	87435.39	18.75	0.000
dry above-ground plant biomass (g)	20.96	3	6.99	32.50	0.000
mainstem height (cm)	38538.36	3	12846.12	75.04	0.000
# of flowers	26212.14	3	8737.38	4.86	0.003
total dry flower weight (mg)	11754682.60	3	3918227.55	23.51	0.000
floral height (mm)	1327.96	3	442.65	153.44	0.000
floral width (mm)	653.61	3	217.87	120.62	0.000
floral depth (mm)	1895.78	3	631.93	197.11	0.000
floral area (h*w) (mm^2)	639250.46	3	213083.49	178.81	0.000

ANOVA indicates that *C. heterophylla* and *C. concolor* were similar, and both were larger than *C. tinctoria,* which was larger than *C. verna (Tables* 3, 4). *Collinsia verna* had the largest number of flowers, but the smallest total dry floral weight, the shortest mainstem, the least amount of dry above-ground plant biomass, but a floral display size equal to that of *C. heterophylla a*nd *C. concolor (Table* 4). *Collinsia concolor* had the highest autonomy rate (Table 4).

Table 4: Mean Values for Each Variable Measured Standard error is reported in the parentheses below the mean. *Collinsia heterophylla B* (highlighted) was used in the among-population analyses (first 3 columns) as well as in the among-species analyses (last four columns). Populations that were not significantly different in ANOVA post hoc tests (Tukey's test) are noted by superscripts of the same letter (a-b, first three columns compared). Species that were not significantly different in ANOVA post-hoc analyses (Tukey's test) are noted by superscripts of the same letter (c-e, last four columns compared).

variable	*C. heterophylla A*	*C. heterophylla C*	*C. heterophylla B*	*C. concolor*	*C. tinctoria*	*C. verna*
autonomy rate	0.10 (0.02)^{a}	0.16 (0.02)^{a}	0.15 (0.02)^{ac}	0.31 (0.05)^{e}	0.10 (0.02)^{cd}	0.15 (0.01)^{cd}
# of branches	13.04 (1.36)^{b}	6.92 (0.54)^{a}	6.06 (0.63)^{ac}	6.13 (1.04)^{c}	12.53 (0.75)^{d}	8.06 (0.47)^{c}
vegetative display size (cm)	287.30 (20.60)^{b}	184.52 (10.20)^{a}	171.78 (12.88)^{ac}	158.76 (25.95)^{c}	252.35 (12.29)^{d}	147.68 (7.49)^{c}
dry above-ground plant biomass (g)	1.38 (0.06)^{b}	1.24 (0.07)^{ab}	1.08 (0.09)^{ac}	0.91 (0.12)^{cd}	1.66 (0.09)^{d}	0.74 (0.03)^{e}
mainstem height (cm)	57.55 (2.62)^{b}	59.40 (2.40)^{ab}	66.43 (2.68)^{ac}	56.10 (6.61)^{cd}	45.63 (2.18)^{d}	23.90 (1.28)^{e}
# of flowers	142.14 (7.03)^{b}	100.28 (5.10)^{a}	94.46 (7.13)^{ac}	93.00 (11.56)^{cd}	97.96 (5.58)^{c}	125.40 (7.04)^{d}
total dry flower weight (mg)	1104.45 (66.72)^{a}	1291.05 (93.63)^{a}	1140.61 (88.67)^{a}	641.74 (62.34)^{cd}	798.33 (81.46)^{c}	370.02 (41.32)^{d}
floral height (mm)	16.74 (0.28)^{a}	17.25 (0.28)^{a}	17.63 (0.32)^{ac}	18.16 (0.68)^{c}	11.82 (0.26)^{d}	10.65 (0.19)^{d}
floral width (mm)	13.00 (0.32)^{b}	14.72 (0.19)^{a}	14.54 (0.21)^{ac}	13.57 (0.32)^{c}	10.44 (0.27)^{d}	9.34 (0.12)^{d}
floral depth (mm)	18.89 (0.44)^{b}	22.49 (0.21)^{a}	22.62 (0.22)^{ac}	18.94 (0.50)^{c}	17.43 (0.40)^{d}	13.29 (0.13)^{d}
floral area (h*w) (mm^2)	217.76 (6.71)^{b}	254.21 (5.68)^{a}	257.36 (7.07)^{ac}	246.95 (12.47)^{c}	125.65 (6.07)^{d}	100.18 (2.75)^{d}

I found no significant correlation between flower size and flower number in these species, suggesting there is no tradeoff in allocation to floral size versus number. For all species, I found significant correlations among the floral size traits: floral height,

width, depth, and area as well as a significant correlation between total dry floral weight and floral width (Table 5). In *C. tinctoria* there were also significant correlations of total dry floral weight with floral depth, height, and area. In *C. tinctoria* I found a significant positive correlation between autonomy rate and floral depth, height, width, and area (Table 5). In *C. verna* I found a significant correlation between autonomy rate and flower number, flower depth, and total dry floral weight (Table 5).

Table 5: Correlations Coefficients Among Species for *C. tinctoria* and *C. verna* with p-values in parentheses. Pearson's correlation results above the diagonal, and Spearman's correlation results below the diagonal. Sample sizes are noted in Table 1. Among species correlation comparisons include values of *C. heterophylla B* (Table 7). Low sample size of *C. concolor* prevented correlational analysis.
* p<0.05, ** p<0.01

C. tinctoria	autonomy rate	flower number	Average floral depth	average floral height	average floral width	average floral area	total dry floral weight
autonomy rate	1	-0.253 (0.097)	0.328* (0.024)	0.450** (0.003)	0.356* (0.021)	0.440** (0.004)	0.150 (0.389)
flower number	-0.267 (0.08)	1	0.008 (0.958)	0.127 (0.424)	-0.066 (0.679)	0.022 (0.892)	0.778** (0.000)
average floral depth	0.196 (0.214)	0.102 (0.519)	1	0.752** (0.000)	0.908** (0.000)	0.923** (0.000)	0.527** (0.001)
average floral height	0.408** (0.007)	0.081 (0.609)	0.549** (0.000)	1	0.692** (0.000)	0.895** (0.000)	0.585** (0.000)
average floral width	0.430** (0.004)	-0.040 (0.800)	0.656** (0.000)	0.514** (0.000)	1	0.935** (0.000)	0.408* (0.015)
average floral area	0.448** (0.003)	0.018 (0.912)	0.664** (0.000)	0.912** (0.000)	0.787** (0.000)	1	0.547** (0.001)
total dry floral weight	0.044 (0.804)	0.836** (0.000)	0.399* (0.018)	0.389* (0.021)	0.158 (0.364)	0.317 (0.064)	1

Table 5 continued

C. verna	autonomy rate	flower number	Average floral depth	average floral height	average floral width	average floral area	total dry floral weight
autonomy rate	1	0.456** (0.001)	0.351* (0.018)	0.117 (0.224)	0.092 (0.550)	0.160 (0.293)	0.303 (0.057)
flower number	0.537** (0.000)	1	0.117 (0.432)	0.266 (0.071)	0.115 (0.443)	0.232 (0.117)	0.584** (0.000)
average floral depth	0.292* (0.052)	0.233 (0.115)	1	0.579** (0.000)	0.587** (0.000)	0.644** (0.000)	0.134 (0.404)
average floral height	0.258 (0.087)	0.335* (0.021)	0.506** (0.000)	1	0.579** (0.000)	0.921** (0.000)	0.245 (0.123)
average floral width	0.102 (0.505)	0.126 (0.399)	0.605** (0.000)	0.536** (0.000)	1	0.846** (0.000)	0.381 * (0.014)
average floral area	0.191 (0.208)	0.295* (0.044)	0.609** (0.000)	0.902** (0.000)	0.822** (0.000)	1	0.343* (0.028)
total dry floral weight	0.474** (0.002)	0.731** (0.000)	0.342* (0.028)	0.449** (0.003)	0.291 (0.064)	0.464** (0.002)	1

Among population variation in floral traits: C*ollinsia heterophylla* populations *B* and *C* were not significantly different from each other in floral size traits, but both differed significantly from population *A*, which produced larger numbers of smaller flowers, and had more branches which created a larger vegetative display size (Tables 4, 6).

Table 6: ANOVA Results Among Populations

variable	type III sum of squares	degrees of freedom	mean square	F	p-value
autonomy rate	0.04	2	0.02	2.32	0.10
# of branches	802.23	2	401.12	19.29	0.00
vegetative display size (cm)	223990.43	2	111995.21	17.87	0.00
dry above-ground plant biomass (g)	1.24	2	0.62	3.37	0.04
mainstem height (cm)	1380.78	2	690.39	3.23	0.04
# of flowers	34077.99	2	17039.00	13.38	0.00
total dry flower weight (mg)	585581.89	2	292790.95	1.17	0.32
floral height (mm)	11.65	2	5.83	1.91	0.15
floral width (mm)	50.95	2	25.48	14.59	0.00
floral depth (mm)	251.26	2	125.63	50.71	0.00
floral area (h*w) (mm^2)	27144.07	2	13572.03	9.62	0.00

Population *A* exhibited a significant correlation between flower number and floral width (Table 7). No other significant correlations between flower size and number were seen, suggesting that in general there are no tradeoffs in flower size and flower number (Table 7). No significant correlations between autonomy rate and any floral trait were detected in any of the *C. heterophylla* populations.

Table 7: Correlation Coefficients Among Populations of *C. heterophylla*. Pearson's correlation results above the diagonal, and Spearman's correlation results below the diagonal. All correlations of autonomy rate and floral measures were non-significant (p>0.05). Sample sizes are noted in Table 1. * p<0.05, ** p<0.01

C. heterophylla A	**autonomy rate**	**flower number**	**Average floral depth**	**average floral height**	**average floral width**	**average floral area**	**total dry floral weight**
autonomy rate	1	-0.179 (0.424)	-0.235 (0.293)	0.074 0.742	-0.327 (0.138)	-0.225 (0.315)	-0.439* (0.46)
flower number	-0.061 (0.786)	1	0.077 (0.733)	0.120 (0.596)	-0.040 (0.861)	0.046 (0.840)	0.549** (0.010)
average floral depth	-0.188 (0.402)	0.205 (0.360)	1	0.247 (0.233)	0.819** (0.000)	0.799** (0.000)	0.701** (0.000)
average floral height	0.128 (0.570)	0.113 (0.617)	0.228 (0.273)	1	0.081 (0.701)	0.612** (0.001)	0.172 (0.443)
average floral width	-0.319 (0.148)	0.118 (0.601)	0.808** (0.000)	0.125 (0.553)	1	0.836** (0.000)	0.540** (0.010)
average floral area	-0.166 (0.461)	0.057 (0.801)	0.771** (0.000)	0.606** (0.001)	0.818** (0.000)	1	0.541** (0.009)
total dry floral weight	-0.389 (0.082)	0.448* (0.042)	0.740** (0.000)	0.203 (0.366)	0.600** (0.003)	0.658** (0.001)	1

Table 7 continued

C. heterophylla B	**autonomy rate**	**flower number**	**Average floral depth**	**average floral height**	**average floral width**	**average floral area**	**total dry floral weight**
autonomy rate	1	0.166 (0.522)	-0.058 (0.751)	-0.189 (0.292)	0.066 (0.717)	-0.087 (0.632)	-0.001 (0.994)
flower number	0.145 (0.421)	1	0.012 (0.949)	0.019 (0.915)	-0.463** (0.007)	-0.206 (0.250)	0.884** (0.000)
average floral depth	-0.085 (0.637)	-0.082 (0.649)	1	0.398* (0.018)	0.143 (0.411)	0.356* (0.036)	0.249 (0.162)
average floral height	-0.100 (0.579)	0.057 (0.751)	0.320 (0.061)	1	0.394* (0.019)	0.884** (0.000)	0.129 (0.473)
average floral width	0.107 (0.503)	-0.402* (0.020)	0.227 (0.189)	0.372* (0.028)	1	0.775** (0.000)	-0.361* (0.039)
average floral area	-0.001 (0.994)	-0.196 (0.275)	0.318 (0.063)	0.793** (0.000)	0.834** (0.000)	1	-0.083 (0.645)
total dry floral weight	-0.041 (0.821)	0.907** (0.000)	0.124 (0.493)	0.109 (0.546)	-0.342 (0.052)	-0.123 (0.496)	1
C. heterophylla C	**autonomy rate**	**flower number**	**Average floral depth**	**average floral height**	**average floral width**	**average floral area**	**total dry floral weight**
autonomy rate	1	-0.022 (0.890)	0.040 (0.809)	-0.097 (0.556)	-0.277 (0.088)	-0.233 (0.154)	-0.011 (0.946)
flower number	-0.073 (0.656)	1	-0.125 (0.449)	-0.114 (0.488)	-0.040 (0.810)	-0.109 (0.509)	0.864** (0.000)
average floral depth	-0.051 (0.758)	-0.063 (0.704)	1	0.282 (0.078)	0.119 (0.466)	0.283 (0.077)	0.072 (0.667)
average floral height	-0.099 (0.547)	-0.118 (0.474)	0.276 (0.085)	1	0.141 (0.386)	0.827** (0.000)	0.075 (0.654)
average floral width	-0.289 (0.075)	-0.035 (0.832)	0.080 (0.622)	0.159 (0.327)	1	0.669** (0.000)	0.086 (0.608)
average floral area	-0.231 (0.156)	-0.130 (0.429)	0.264 (0.100)	0.844** (0.000)	0.623** (0.000)	1	0.096 (0.567)
total dry floral weight	0.001 (0.993)	0.901** (0.000)	0.169 (0.312)	0.076 (0.651)	0.094 (0.574)	0.062 (0.712)	1

Discussion

Among-species scaling in the genus *Collinsia*: Species within the genus *Collinsia* exhibit a wide range of floral sizes (6-16 mm Figure 1). While there is a general positive relationship between flower and plant height within the genus (Figure 4), many species do not conform to this relationship. The vegetatively-largest species have, in general, large flowers, and the vegetatively smallest species vary significantly in flower size (Figures 4). Surprisingly, the two species with the smallest vegetative size are both the largest and smallest flowered species in the genus (*C. corymbosa* and *C. torreyi wrightii,* respectively). This variation is also reflected in the dramatic changes in rank order of plant size and flower size (Figure 3).

The allometric scaling approach used here accounts for variation in plant size among related species when considering variation in floral size. The regression of average floral height on average plant height explained 20% of the variation among these species, indicating that deviation from the general scaling relationship within *Collinsia* is common. The degree and direction of deviation from the general scaling relationship can suggest differences in the selective environment in nature that affect flower size. Species that fall in the upper left quadrant of Figure 4 (*C. corymbosa, C. greenei, C. bartisiifolia var. bartisiifolia*) have larger flowers than expected by the allometric scaling relationship. This suggests that these species are allocating more resources than expected to the floral traits associated with pollinator attraction (corolla size). For example, *C. corymbosa* has the largest floral size and the second smallest plant size of all the *Collinsia* species. Interestingly, *C. corymbosa* lives on the nutrient poor sand

dunes of Monterey County, California, where it is endemic. The over-allocation to flower size seen in this species suggests that large flowers are favored even though they are expected to be costly, likely because they increase pollinator attraction and may increase outcross pollen receipt.

In contrast, species that fall in the lower left quadrant of Figure 4 have significantly smaller flowers than expected. These include *C. torreyii var wrightii, C. parviflora, C. rattanii, and C. sparsiflora var sparsiflora*. These species are expected to have been under selection to reduce floral size and are likely highly selfing. *Collinsia rattanii* is found in open coniferous forests in northwestern USA while *C. parviflora* is found on rock-outcrops, grassy slopes, and beaches from California north to British Columbia and east to Ontario (Parachnowitsch and Elle 2004) as well as moist shady places in the mountains (Neese 1993). Elle and Carney (2003) showed that while pollinators do occasionally visit *C. parviflora*, they preferentially visit large-flowered individuals within populations and larger-flowered populations over smaller flowered populations. *Collinsia parviflora* has been shown to have high autonomous selfing rates and small-flowered individuals produce significantly more seeds through self-fertilization in a natural pollination environment than larger-flowered individuals (Elle 2004).

Surprisingly there are no species that fall in the lower right quadrant, and few that fall significantly above the regression line in the upper right quadrant. In fact, the amount of variation around the regression line significantly decreases as plant size increases, suggesting either a genetic constraint on the production of larger or smaller flowers on large plants, or that there has been no selection to produce large plants with smaller flowers, or large plants with very large flowers. One explanation for this low variation at

large plant size may be that species that inhabit productive environments can acquire enough resources to produce large plant sizes, and are not likely to experience selection pressure to reduce floral costs. Species that inhabit highly productive environments with pollinator variability or failure may not be selected to reduce floral size, but may instead be selected to change the timing of selfing via reduced herkogamy and dichogamy to ensure reproductive success. The reduced variability in floral size in the species with the largest plant sizes may also indicate an optimum in floral size for larger plants.

No clear predictions can be made about the mating system of species that fall along the regression line except that small flowered species are likely to be more selfing while large flowered species are likely to be more outcrossing. For example, *C. verna*, with an average floral height of 15 mm is among the largest flowers in the genus. This species exhibits a delayed selfing mating system where flowers are able to outcross first, but reduce herkogamy late in floral life to enable self-fertilization (Kalisz et al 1999.) In this manner, ovules that are not outcross-fertilized can be self-fertilized yielding a mixed mating system. *Collinsia verna* experiences pollinator variability within and among seasons (Kalisz and Vogler 2003), and expresses a mixed mating system with outcrossing rates dependent on pollinator visitation rates (t ranges from 0.62 to 1.0; Kalisz and Vogler 2003; Kalisz et al 2004). Likewise, *C. heterophylla,* also among the largest flowered species, has been shown to expressa range of outcrossing rates (t ranges from 0.32 to 0.64; Mayer, et al 1996).

The availability of a phylogeny for the tribe *Collinsieae* (Armbruster et al 2002) and the use of allometric scaling and the comparative method allow for several interesting

patterns to be explored. First, there are three species whose subspecies fall both above, on, and/or below the line (Figure 4) *(C. sparsiflora, C. torreyi, and C. bartsiifolia).* In the subspecies of *C. sparsiflora* all have similar average plant height, but express large variation in floral size - one subspecies falls above (*C. sparsiflora arvensis 17 mm*), one falls along (*C. sparsiflora collina 10 mm*), and one falls below *(C. sparsiflora sparsiflora 7mm)* the regression line. These three subspecies are likely experiencing different selection pressures on floral size and may be evolving toward different mating systems. *Sparsiflora arvensis* inhabits dry meadows, old fields, and rocky grass slopes (Neese 1993) and may experience an environment in which pollinators are more abundant and more dependable and may be under selection pressure to increase floral size and outcrossing rate. In contrast*, C. sparsiflora sparsiflora* is generally found in grassy disturbed areas and in chaparral (Neese 1993) and may experience an environment in which pollinators are rare or unpredictable and experience selection pressure to reduce floral size and to increase autonomy ability to ensure that offspring are produced (reproductive assurance). *Collinsia sparsiflora collina* is found in a wider range of disturbed habitats including roadsides, grassy fields, open chaparral, and foothill wetlands (Neese 1993). This variety of environments may favor delayed selfing. In contrast, the four subspecies of *C. torreyi* all have similar plant sizes, but one of the four (*C. torreyi wrightii*) has a significantly smaller average flower size (5 mm) than the others (7.5mm) (Neese 1993). *Collinsia torreyi wrightii* inhabits the highest elevations of all the subspecies and the change in allometric scaling may parallel a change in mating system in response to low pollination and resource conditions related to a short growing season. The styles of the smaller flowered *C. torreyi wrightii* and *C. sparsiflora*

sparsiflora come into contact with self-pollen earlier in floral development than the larger flowered subspecies (Armbruster et al 2002) further supporting the hypothesis that the smaller subspecies may be evolving towards a more selfing mating system.

Allometric scaling within and among four species of *Collinsia*: A closer look at the scaling relationships within species from my greenhouse experiment (Figures 6 and 6) reveals individual variation not possible to see in the among-species level analyses (Figure 4). The scaling coefficients (k) differ among species analyzed, and the k coefficients for the species differ from that calculated for genus overall suggesting evolutionary divergence among species. While species overall occupied different areas of the scaling graph (Figures 6a, b), individuals overlap among species. Since, individuals in this experiment were grown under identical greenhouse conditions, this suggests that they differ genetically in their individual responses to the growth environment. Individual variation in plant height and floral height for *C. verna, C. heterophylla A, and C. heterophylla C* was extreme and the regression was not significant for any of these species.

Variation in floral size within and among species of *Collinsia*: Many factors can cause flower size to become smaller, including resource limitation (Holtsford and Ellstrand 1992; Diggle 1997; Elle and Hare 2002; Case and Barrett 2004) and pollinator limitation (Elle and Carney 2003) or flower size to become larger, such as increased pollinator attraction and/or competition for pollinators (Conner and Rush 1996; Totland 2001). In both the among and within species comparisons, the choice of variables to

represent flower size led to very different scaling relationships and different patterns of species variation (Figures 6ab, 6ab). For example, in my ANOVA analyses, I found no difference among populations of *C. heterophylla* in floral height, but significant differences were found in floral width, depth, and area (Table 4). When I used floral area (height *width of the corolla) instead of floral height in my regressions, I marginally increased the variation explained for *C. heterophylla B* from 10% to 13%. Since no single measurement was found to be used consistently in the literature, several variables were measured in this study for both plant and flower size. Correlation analyses among these variables showed no consistent pattern among species or populations. In *C. tinctoria* and *C. verna* there were significant correlations among the floral size measures (Table 5), but *C. heterophylla* varied in its correlations for each of the different populations (Table 7). Significant correlations of flower size to total dry floral weight were found in *C. tinctoria,* but not in the other species studied. The amount of variation in floral trait estimation, and in the correlations among traits, makes it difficult to determine the 'best' measures to use in these analyses. My results suggest that the choice of variables in allometric scaling studies can affect the results and that multiple measurements should be taken to fully understand scaling patterns.

Floral height is less variable than plant height (Figure 5), and has a lower coefficient of variation (CV) across all species. This suggests that natural selection may be acting differently on the two variables- maintaining floral size while allowing plant size to vary with environmental conditions. Previous field estimates of average plant (main stem) height in *C. heterophylla* varied from 210 to 270 mm (Weil and Allard 1967). My greenhouse estimates of height for C. heterophylla ranged from 310-355 mm (Block 1)

to 575 to 665 mm (Block 2) suggesting that plant height is indeed a flexible character and can vary with changing environmental conditions.

Collinsia heterophylla and *C. tinctoria* are among the largest-flowered species in the genus, (Figure 1) and there is clearly more variation in floral size for the larger-flowered species than for the smaller-flowered species. Interestingly, some species were so variable in floral size despite constant plant size that they have been differentiated into varieties (C. sparsiflora, C. bartsiifolia, and C. torreyi) (Neese 1993).

When I compare the results of this study to previous work on *Collinsia*, I find that estimates of floral size in *C. heterophylla* are more variable among populations than within populations. This might indicate stabilizing selection within populations, but divergence among populations. Previous *C. heterophylla* estimates of average corolla lobe width varied from 5-6 mm and average corolla lobe length varied from 7.6-10.6 mm (Charlesworth and Mayer 1995). My estimates of *C. heterophylla* average corolla height varied between 16.7 and 17.6 mm and average corolla width averaged 13-14.5 mm. In block 1, where temperatures were significantly higher, average floral height ranged from 14-15 mm and width ranged from 10-12 mm. While block 1 estimates are smaller than block 2 estimates, both blocks are larger than previous reports. There were no significant correlations between flower size and flower number in any of the species investigated here, suggesting there is no tradeoff in allocation to size versus number.

Autonomy ability (= the production of fruits via autonomous self pollination) is also variable within and among these species. Populations of *C. heterophylla* autonomy rates averaged 0.10 to 0.16 while individuals varied from 0 to 0.5. Since all plants were

grown in greenhouse conditions with regular water and fertilizer, they should not have been resource limited. *Collinsia heterophylla* is found throughout California (Neese 1993) and populations appear to vary significantly in their ability to autonomously self-fertilize (Armbruster et al 2002). This level of variation is found in other species as well, and may not be simply described by population level differences. In one study of *C. verna*, average autonomy rates were estimated at 0.33, with individual estimates varying from 0 to 0.8. In a second study, *C. verna* populations were estimated to have average autonomy rates of 0.5 with individual estimates varying from 0 to 1.0 (Kalisz and Vogler 2003). The average autonomy rate estimated here for C. verna was lower (0.15) and individuals varying from 0 to 0.35. One explanation for the difference is that previous studies were done in exclosures under field conditions, while this study was conducted under greenhouse conditions. It is possible that wind may facilitate within flower selfing. It is also possible that given the degree of individual variation in autonomy ability, that my estimates may simply be a result of sampling.

In *C. tinctoria*, autonomy rate was significantly positively correlated with all measures of individual flower size (floral height, width, depth, and area; Table 5) suggesting that floral size and shape are important factors in the ability of individual flowers to produce seeds autonomously. It is possible that either herkogamy and/or dichogamy are influencing the low average selfing ability in this species, and that changes in floral size and shape may enable self-fertilization among some individuals. In contrast, autonomy rate in *C. verna* was significantly correlated with the total number of flowers on a plant as well as the total dry floral biomass (Table 5). In this species, autonomy ability is not

correlated to size and shape variables, but instead is correlated to the total number and weight of flowers.

Conclusions

The genus *Collinsia* is variable within and among species in morphological characteristics related to flower size and plant size. The degree and direction of variation from the general allometric scaling pattern can be used to further examine this variation and to generate hypotheses concerning the selective environments that may be influencing the observed variation. Scaling relationship for flower size and plant size differ at the genus, species, and population level and, in some cases, are non-significant. In addition to floral size, autonomy ability was found to vary. Both the phylogenetic history and the selective environment will have a significant effect on deviations from the allometric relationships of a population or species and may affect the mating system expressed. Additional work is in progress to further understand the forces generating variation within this genus and to understand the potential role of mating system flexibility in the evolution of species.

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