# SELECTION ON FLORAL DESIGN IN POLEMONIUM BRANDEGEEI (POLEMONIACEAE): FEMALE AND MALE FITNESS UNDER HAWKMOTH POLLINATION

Mason W. Kulbaba<sup>1,2</sup> and Anne C. Worley<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada <sup>2</sup>E-mail: umkulbam@cc.umanitoba.ca

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Plant-pollinator interactions promote the evolution of floral traits that attract pollinators and facilitate efficient pollen transfer. The spatial separation of sex organs, herkogamy, is believed to limit sexual interference in hermaphrodite flowers. Reverse herkogamy (stigma recessed below anthers) and long, narrow corolla tubes are expected to promote efficiency in male function under hawkmoth pollination. We tested this prediction by measuring selection in six experimental arrays of *Polemonium brandegeei*, a species that displays continuous variation in herkogamy, resulting in a range of recessed to exserted stigmas. Under glasshouse conditions, we measured pollen removal and deposition, and estimated selection gradients (β) through female fitness (seeds set) and male fitness (siring success based on six polymorphic microsatellite loci). Siring success was higher in plants with more nectar sugar and narrow corolla tubes. However, selection through female function for reverse herkogamy was considerably stronger than was selection through male function. Hawkmoths were initially attracted to larger flowers, but overall preferred plants with reverse herkogamy. Greater pollen deposition and seed set also occurred in reverse herkogamous plants. Thus, reverse herkogamy may be maintained by hawkmoths through female rather than male function. Further, our results suggest that pollinator attraction may play a considerable role in enhancing female function.

KEY WORDS: Herkogamy, Hyles gallii, male fitness, pollen movement, selection gradients.

Floral traits affect mating opportunities because they attract pollinators and/or facilitate the efficient movement of pollen. Quantitative traits such as flower size can be correlated with nectar volume or sugar content, providing a means for pollinators to estimate potential rewards (Bell 1985; Glaettli and Barrett 2008; Kaczorowski et al. 2008). In addition, flower color (Meléndez-Ackerman et al. 1997) and olfactory cues (Raguso 2008) may appeal to specific pollinators or functional groups of pollinators (cf. Fenster et al. 2004). Other aspects of floral design, including flower shape and the positioning of sex organs, typically facilitate efficient pollen transfer but are not necessarily associated with pollinator attraction. These traits orient pollinators, so that pollen

is consistently placed where it can be deposited on the stigmas of subsequently visited flowers rather than consumed or lost (Fenster et al. 2009). Traits such as nectar guides or landing pads affect both attraction and efficiency; phenotypes that enhance both functions may be under stronger selection than those that only increase one aspect of successful pollination. However, we know of no studies that have examined this possibility.

The majority of flowering plants are hermaphroditic, and may simultaneously function as females and males. Therefore, selection on attraction and efficiency may occur through either gender function, and even be in contrast among genders (Campbell 1989; Ashman and Morgan 2004). Indeed, conflicting selection among gender functions has been predicted to be common (Morgan 1992), but evidence for gender conflict is sparse (Delph and Ashman 2006; Sahli and Conner 2011, but see Campbell 1989). Further, <u>Bateman (1948)</u> predicted that selection through male function should be stronger than through female function. This postulate too is controversial, in part because studies examining direct measures of male fitness lag behind those using surrogate measures of siring success (Conner 2006; but for a recent example, see Sahli and Conner 2011). More studies that directly estimate both female and male fitness are required to fully resolve the prevalence of gender conflict, and the relative magnitude of selection through female and male functions in flowering plants.

A variety of traits influence attraction and female fitness in hawkmoth-pollinated species. In natural settings, hawkmoths display an innate attraction to lightly colored flowers (Raguso and Willis 2003) with particular olfactory cues (Riffell et al. 2008). However, the relative roles of corolla color and scent are still unclear (Goyret et al. 2008). Efficient pollen transfer is associated with long, narrow corolla tubes or nectar spurs (Nilsson 1988; Alexandersson and Johnson 2002; Brunet 2009). Long flowers promote stronger contact between long-tongued hawkmoths and floral sex organs because rewards (usually nectar) are produced at the base of the flower or spur. This increased contact often promotes efficient pollen deposition. However, selection by hawkmoths has primarily been detected through female fitness alone, or with indirect estimates of male fitness such as pollen removal or pollinator visitation rates (e.g., Campbell et al. 1997; Sletvold and Ågren 2010; Vanhoenacker et al. 2010). Direct measurements of male fitness are notoriously difficult to obtain, and apparently have never been estimated for hawkmoth-pollinated species. Thus, our understanding of hawkmoth-mediated selection lags behind that of other groups, such as bees and/or hummingbirds, even though hawkmoths are important pollinators across a variety of plant families (Grant 1983; Thomson and Wilson 2008).

One floral trait of particular importance for efficient pollen transfer in hermaphroditic plants is the spatial separation of sex organs, herkogamy. Although herkogamy may reduce selfpollination and subsequent inbreeding depression, it occurs in many species with genetic self-incompatibility (reviewed in Barrett 2003). Therefore, Webb and Lloyd (1986) proposed that herkogamy may function to reduce the interference between female and male functions, limiting the wastage of gametes. In approach herkogamy, the stigma is presented above or beyond the level of the anthers, so that pollinators (and outcrossed pollen) contact the stigma first when visiting a flower (Barrett 2003). In contrast, reverse herkogamy presents the anthers above a recessed stigma, ensuring the anthers are contacted before the stigma. Approach herkogamy is common and associated with a diversity of pollinators. Reverse herkogamy is less common, and it is thought to be associated with long-tongued pollinators, such as hawkmoths, when accompanied by a long, narrow corolla tube (Webb and Lloyd 1986). This association is hypothesized because the long, slender proboscis of a moth would more likely contact a recessed stigma (reverse herkogamy) contained within a narrow tube, than it would an exserted stigma (Barrett 2002). Further, pollen removal should be greater when the anthers are presented above the stigma, because physical interference from the stigma and style would be reduced. However, the functional association between reverse herkogamy and hawkmoth pollination has not been explored empirically.

Surprisingly, causes and consequences of continuous variation in herkogamy in self-incompatible species have received little attention (but see Forrest et al. 2011), even though continuous variation in herkogamy is very widespread. This contrasts with the majority of studies examining selection on herkogamy, which have focused on the 10% of flowering species that display polymorphisms (e.g., heterostyly) in sex-organ arrangement (Barrett et al. 2000; Barrett 2003), or on species in which reduced herkogamy results in selfing (e.g., Fishman and Willis 2007). In outcrossing species, selection may sometimes favor reduced herkogamy if close proximity of stigma and anthers is required to ensure that small pollinators contact both sex organs, as in Raphanus raphanistrum (Sahli and Conner 2011). However, in general, selection should favor a phenotype that will reduce the interference between female and male function, and still allow outcrossed pollen to reach the stigma. Interestingly, stabilizing selection for approach herkogamy occurs in Ipomoea wolcottiana under hawkmoth pollination (Parra-Tabla and Bullock 2005). This result contradicts the expectation of reverse herkogamy in hawkmoth-pollinated taxa outlined above (Webb and Lloyd 1986). Additional effort is required to quantify selection on herkogamy in outcrossing species, and to determine whether and under which conditions lepidopteran pollinators select for reverse herkogamy.

We measured selection on floral traits of the self-sterile, subalpine perennial *Polemonium brandegeei*, under pollination by captive individuals of the hawkmoth *Hyles gallii*. *Polemonium brandegeei* displays tubular corollas that are cream-white in color, and emit a strong, heavy, sweet smell. Populations of *P. brandegeei* exhibit continuous heritable variation in style length, that results in a range of approach (exserted stigma on long style) to reverse (recessed stigma on short style) herkogamy (Kulbaba and Worley 2008). Hawkmoths (*H. lineata*, *H. gallii*) and hummingbirds (*Selasphorus platycercus*) are both pollinators of *P. brandegeei*. We proposed previously that hawkmoths maintain reverse herkogamy in *P. brandegeei* populations (Kulbaba and Worley 2008).

To fully evaluate the interaction between hawkmoths and floral design, we adopted a strategy similar to that suggested by Campbell (2009). We selected individual plants based on herkogamy (approach or reverse) to assemble experimental arrays of *P. brandegeei*, pollinated exclusively by captive *H. gallii* under controlled conditions. This approach allowed manipulation of a single floral trait, whereas the remaining traits were allowed to vary naturally (Campbell 2009). Because visits by hawkmoths occurred in the absence of other visitors, we were able to directly measure hawkmoth-mediated selection on floral design. To our knowledge, this was the first study to directly measure selection by hawkmoths through both female and male fitness.

Our main objective was to assess how floral dimensions and nectar rewards affect attraction, pollen movement, and fitness under hawkmoth pollination. Secondary objectives were to describe hawkmoth foraging behavior on and among inflorescences of P. brandegeei, and to compare selection through female and male fitness. We expected that (1) hawkmoths would be more attracted to plants with greater rewards (larger flowers corresponding to higher nectar volume and/or sugar content), but would not respond to variation in corolla shape or sex-organ positioning (floral design). Rather, (2) we expected variation in floral design to affect male and female fitness through effects on efficient pollen removal and deposition. Specifically, we predicted that (3) hawkmoths would select for long and narrow corollas displaying reverse herkogamy, corresponding to the flowers of other hawkmoth-pollinated taxa (Nilsson 1988; Strakosh and Ferguson 2005; Kulbaba and Worley 2008).

### Materials and Methods FLORAL TRAITS AND EXPERIMENTAL DESIGN

To generate experimental populations (hereafter referred to as "arrays") of *P. brandegeei* flowers, we grew individual plants in Premier Pro Mix<sup>®</sup> medium with Osmocote Plus<sup>®</sup> slow-release fertilizer (10:10:10) in 800 mL Deepots<sup>®</sup>. We raised the plants in a greenhouse (temperature range:  $23-27^{\circ}$ C) under a 16-h photoperiod, with seeds obtained from Lone Tree Gulch, Southern Colorado ( $38^{\circ}30'17''$ N,  $107^{\circ}11'26''$ W).

Plants were arrayed as in Figure 1. This arrangement produced an even distribution of approach and reverse herkogamous plants, and an equal probability of a hawkmoth encountering an approach or reverse herkogamous plant on entering the array. Arrays consisting of 12–18 plants each (depending on available phenotypes) were placed in a controlled environmental flight chamber (Pernal and Currie 2001) with a 12-h diurnal period, including a 30 min simulated sunrise and sunset "illumination" and "dimming" period, respectively. Daytime temperature was 24°C, and nighttime temperature was 17°C with a relative humidity of ca. 60%. Pupae of *H. gallii*, a known pollinator of *P. brandegeei*, were obtained from Worldwide Butterflies (Cornwall, UK) and covered with damp peat moss until emergence as flying adults. We introduced two to three adult *H. gallii* to the same flight chamber



**Figure 1.** Arrangement of approach (A) and reverse (R) herkogamous plants in the experimental array populations. Distance between plants was 45 cm.

containing the array plants, and allowed them to forage on the plants for 48 h. A total of six arrays (94 plants), and 14 individual *H. gallii* adults were used for this study. Arrays were run consecutively because we had only a single flight chamber, and unique hawkmoths were used in each array. Captive *H. gallii* were similar in both mass and proboscis length to those from natural populations.

We chose plants from different maternal families (seed source plants no closer than 1.5 m) based on their herkogamous phenotype for inclusion in an array (total of six arrays). Herkogamy was directly measured as the position of the stigma relative to the nearest anther (Fig. 2). However, because stigma–anther separation is a continuous trait, our array populations contained a range of phenotypes (reverse herkogamy: approximately -4.0 to -0.2 mm; approach herkogamy: approximately 0.2-4.6 mm),



**Figure 2.** Diagram of floral measurements. Dashed line indicates style position of an approach herkogamous flower, and a solid line indicates style of a reverse herkogamous flower.

which was comparable to the range seen in natural populations. Inflorescences were standardized to seven freshly opened flowers, also to reflect the natural display size (mean of 6.97, n = 369 across three natural populations; Kulbaba and Worley 2008). Thus, 84–126 flowers were available to hawkmoths in each array.

We measured additional floral traits to determine how floral design affects female and male fitness in P. brandegeei. These traits were corolla tube length, corolla tube width at the base and apex of the flower, height of sex-organ presentation relative to the base of the flower, flower mass (dry weight of corolla, calyx, androecium, and gynoecium), nectar volume, and nectar sugar content (Fig. 2). Floral traits were selected for detailed examination because they had a large influence on phenotypic variation in floral dimensions and/or seemed likely to affect the attractiveness of flowers, or pollen transfer efficiency by hawkmoths. Further, floral dimensions were heritable in a greenhouse crossing experiment, and could potentially respond to selection (Kulbaba and Worley 2008). We obtained floral dimensions and nectar measurements just before the extra flowers were removed to standardize flower number, so that measured flowers were a similar age to flowers visited by hawkmoths. Floral dimensions were measured on flowers used in the array and were made to the nearest 0.1 mm with digital calipers. Nectar measurements were destructive and were therefore made on flowers that had been removed. Polemonium brandegeei is weakly protandrous, with stigmas becoming receptive 6-24 h after anthers dehisce. All flowers visited by hawmoths in our arrays were functionally hermaphroditic, with dehisced anthers and mature stigmas.

Nectar volume was determined by removing the nectar with calibrated 2 µl micropipettes (Drummond Scientific, Broomall, PA). Nectar was removed from unvisited flowers that were on the same inflorescence as the experimental flowers. These samplings were taken as representative of the inflorescence because a pilot study in natural populations showed greater variation in nectar volume among plants than within plants. The extracted nectar was dried on clean filter paper for later quantification of sugar content. We determined the sugar concentration of extracted nectar via the colorimetric anthrone technique (McKenna and Thomson 1988). We performed two replicate analyses of the same nectar sample and used the mean (Kearns and Inouye 1993). Finally, we compared the nectar sugar content of the array plants with 10 samples from Taylor Canyon, Colorado (39°34'33"N, 104°22'26"W) to see if greenhouse and field plants differed with respect to floral reward. Seed from Lone Tree Gulch, Colorado was unavailable due to a fire at this population. Taylor Canyon, Colorado was the closest population of P. brandegeei resembling Lone Tree Gulch, in elevation and general habitat features (Kulbaba and Worley 2008).

#### HAWKMOTH BEHAVIOR AND FITNESS ESTIMATES

Pollinator behavior was observed by recording H. gallii foraging bouts with a video camera. Each bout consisted of consecutive visits to one or more flowers; bouts began when a hawkmoth visited the first flower and ended when the hawkmoth exited the array and rested without flight for at least 5 min, or ceased visiting flowers. The number of visits to each plant was recorded, as was the sequence of plants visited within each H. gallii foraging bout, to determine if hawkmoths display a preference while foraging. Because pollinator movements within an inflorescence can indicate the potential for geitonogmous pollen transfer, we recorded whether moths moved to higher or lower flowers while foraging within an inflorescence. We scored the total number of flowers visited/probed for each plant ("flowers visited per plant"), and the number of times an individual plant was visited over the 48-h array period ("visits per plant"). Each visit to a plant could include probes of multiple flowers. Unfortunately, we were unable to record flower-handling time as we could not consistently observe flower entry and exit given the camera angle. However, we did record the total length of each foraging bout. Therefore, we calculated the average time spent per flower by dividing the length of an individual foraging bout by the total number of flowers visited in that bout. Finally, we observed the location of pollen on the bodies of hawkmoths with the aid of a stereomicroscope.

After the standardized foraging period, the plants were returned to the greenhouse and allowed 24 h for ovule fertilization. Pilot experiments had shown 24 h to be sufficient for fertilization (M. W. Kulbaba, unpubl. data). We then collected the anthers and stigmas from five of the seven experimental flowers. We stored anthers in 70% ethanol, and later determined the number of pollen grains remaining with a Multisizer 3 particle counter (Beckman-Coulter, Fullerton, CA). We mounted stigmas on fuchsin jelly slides (Beattie 1971), and manually counted the number of pollen grains deposited on the stigma from digital images (Image-pro express, Media Cybernetics Inc., Bethesda, MD). We were unable to distinguish between self and outcross pollen; therefore counts of pollen deposition reflect both combined.

We measured fitness through both female and male functions. Female fitness for individual plants was the number of seeds set. Male fitness was the number of seeds sired by each plant. Offspring from each array were raised in a greenhouse to the seedling stage under similar conditions to the array plants. We extracted DNA from dried leaf tissue and genetically screened both parents and offspring from each array (up to five offspring per array plant) with six polymorphic microsatellite loci as described in Kulbaba and Worley (2011). Microsatellite genotypes were manually scored with genemapper software 4.0 (Applied Biosystems, Foster City, CA) for parents and offspring, and then analyzed with the program Cervus version 3.03 (Kalinowski et al. 2007) to determine the most likely paternal parent. Cervus uses a maximum-likelihood approach (Meagher 1986) based on Mendelian segregation probabilities of parent and offspring genotypes to assign parentage. These probabilities are first determined through a simulation performed by Cervus version 3.03. The simulation parameters were as follows: 10,000 cycles, complete sampling of 17–11 candidate fathers (reflecting the array size minus the known mother because *P. brandegeei* is self-sterile), 0.750 as the proportion of loci typed, 0.01 as the rate of typing error (calculated from repeat genotyping), 0.95 for the strict confidence level, and 0.80 for the relaxed confidence level because we did not have to consider pollen donors outside our closed experimental populations (cf. Nishizawa et al. 2005; Hodgins and Barrett 2008a).

#### STATISTICAL ANALYSIS

We used general linear models employing analysis of covariance (ANCOVA; Proc GLM and Proc GENMOD) to assess factors influencing hawkmoth behavior, patterns of pollen transfer, and fitness measures. Array number was included in all ANCOVAs as a fixed factor. Both two- and three-way interactions among the explanatory variables (fixed effect and covariates) were analyzed, as were quadratic terms to explore the possibility of stabilizing or disruptive selection. Nonsignificant interactions and effects were removed from the model in a reverse stepwise fashion (Sokal and Rohlf 1995). This general approach was used in all subsequent analyses involving multiple explanatory variables. We also examined the consistency of trends found with ANCOVA by conducting multiple regressions on each array. All trends and relationships described were significant in at least three of the six replicate arrays, and were consistent in all replicate arrays (i.e., the signs of slope estimates were consistent).

In many analyses, more than one explanatory variable was significant. To best portray the influence of specific covariates on dependent variables, we adjusted the data in our figures to highlight the effects of statistically significant floral dimensions. Predicted values were first generated with partial regression coefficients and observed values of the focal covariate. The partial regression coefficients and mean values of the remaining significant covariates were also included in the calculation of values. Residuals from the mixed model were then added to the predicted values to generate adjusted values (cf. Worley and Harder 1996). All analyses and generation of adjusted values were performed in SAS 9.1.2 (SAS Institute 2004).

#### Hawkmoth behavior

We analyzed hawkmoth behavior to assess pollinator preferences. The sequence of plants visited, number of times a plant was visited, and the mean number of flowers visited per plant were treated as dependant variables in general linear models (Proc GLM). Plants unvisited in a bout were treated as missing data, and not included in calculation of mean number of flowers visited per plant. All plants within arrays were visited within the 48 h that the arrays were available to hawkmoths. Measured floral traits were included as explanatory covariates. All three dependent variables were square-root transformed and covariates were  $log_{10}$ transformed to improve normality of residuals.

To determine if hawkmoths foraged longer on certain floral phenotypes, we calculated the "average phenotype" of plants visited in a foraging bout. Each plant phenotype was weighted by the proportion of total flower visits that it received in a bout. Average phenotypes were calculated for flower size (mass), herkogamy, and nectar sugar content and volume. The average time spent per flower (total time of foraging bout/total number of flowers visited in that foraging bout) was analyzed as a dependant variable, with the "average phenotypes" included as explanatory factors in a general linear model (Proc GLM).

#### Female and male function

We first sought to identify floral traits important for pollen removal and deposition under hawkmoth pollination. Pollen remaining in anthers and deposited on stigmas were treated as dependent variables after  $log_{10}$  transformation to meet assumptions of normality. Floral traits, number of visits received by a plant, and the mean number of flowers visited per plant were included as explanatory variables.

To detect selection on floral traits under hawkmoth pollination, we examined associations between floral traits and female (seeds set) or male (seeds sired) fitness. The number of seeds set and number of seeds sired were analyzed as dependant variables. Floral traits, number of visits received by a plant, and the mean number of flowers visited per plant were included as explanatory variables (covariates). Seed set was square-root transformed to improve normality, and analyzed with a general linear model (Proc GLM). However, we analyzed the number of seeds sired with a generalized linear model (Proc GENMOD) along a Poisson probability distribution, as the data best followed this distribution. Ideally, we would have included pollen export and import as explanatory variables. However, we were unable to differentiate between self and outcross pollen. Therefore, we individually examined the relationships between seed set and pollen deposition, and siring success and pollen removal.

Selection gradients were calculated as the linear regression coefficients between standardized fitness measures and floral traits. We standardized fitness by dividing individual fitness measures by mean fitness (Lande and Arnold 1983). Explanatory variables (floral traits) were standardized to a mean of one, by dividing individual trait values with the mean trait value (Hereford et al. 2004). Both linear ( $\beta$ ) and quadratic selection gradients ( $\gamma$ ) were examined for all floral traits, to allow for both directional and stabilizing/disruptive selection, respectively. We included the

linear terms of effects in models that included quadratic terms ( $\gamma$ ). Although female fitness measures (seeds set) required squareroot transformation before analysis, we report selection gradient estimates from the untransformed data, because estimates from transformed fitness measures are biased (Lande and Arnold 1983). However, reported *P*-values are from the transformed ANCOVA (Mitchell and Shaw 1993; Caruso 2000). Finally, we used Welch's *t*-test (assuming unequal variance) to statistically examine the relative magnitude of selection gradients on traits through female and male function.

#### Fitness surfaces

To further examine potential multivariate or correlated selection, we analyzed nonlinear multidimensional fitness surfaces. We used the projected pursuit regression described in Schluter and Nychka (1994) to fit spline curves to our fitness data without making assumptions about the shape of the function. A single projection was fit to our multidimensional data, with a smoothing parameter  $(\lambda)$  that minimized the general cross-validation (GCV) score, a measure analogous to least-square difference. This value of  $\lambda$  was chosen after testing a range of smoothing parameters (described in Schluter and Nychka 1994). We conducted separate analyses of relative female, male, and total fitness. Total fitness was the average of relative female and relative male fitness. We used relative female and male fitness to prevent the larger values of seed set from dominating variation in total fitness. Floral traits included as covariates were basal-tube diameter, herkogamy, nectar sugar, and flower mass. These variables were indicated as being potentially important by ANCOVA, or likely correlated with other traits (e.g., mass). The contribution or loading of each floral trait on the fitness measures was calculated, and 95% confidence intervals were generated through bootstrap analysis (1000 replicates).

### Results

Our analyses explicitly assessed the possibility of nonlinear and multivariate relationships between floral traits and either pollinator behavior or plant fitness. However, all interaction and quadratic terms in the ANCOVAs were nonsignificant. These results were mirrored in our projected pursuit regression. Thus, in this study, the effects of each explanatory variable were linear and independent of other variables included in the final models.

#### **POLLINATOR BEHAVIOR**

On average, moths visited 3.7 plants, 3.4 flowers per plant, and 13 flowers in total during each foraging bout. This was a small fraction of the available plants and flowers, given that arrays contained 12-18 plants and seven flowers per plant for a total of 84-126 flowers per array. Over the 48-h duration of an array, hawkmoths visited an average of 194 flowers. All array plants were visited in all six arrays. However, both the number of visits received by each plant and the number of flowers visited per plant varied widely (Appendix A). Interestingly, H. gallii individuals foraged in a "directional" pattern, typically without returning to plants previously visited within a foraging bout. Hawkmoths exited the arrays by flying upwards, and then alighting on the wall of the flight chamber. Within an inflorescence, H. gallii was equally likely to move up or down. We observed a total of 1165 movements between individual flowers across the six replicate arrays, and determined that  $\sim$ 53% of visits (615) proceeded in an upwards direction on an inflorescence, whereas the remaining 47% of visits (550) proceeded in a downward direction ( $\chi^2 = 3.67$  for expectation of equal number of movements, P = 0.075).

We assessed pollinator preferences by analyzing the sequence of plants visited, the number of visits received by each plant, the number of flowers visited per plant, and estimated handling time per flower. Plants with larger flower mass tended to be visited earlier in a foraging bout (Table 1; Fig. 3) but they did not receive more visits overall than plants with smaller flowers (Table 1). Moreover, measures of floral reward (nectar volume, nectar sugar content) were not correlated with flower mass (Appendix B). Contrary to our expectations, the number of visits received by an array plant was not influenced by floral reward,

**Table 1.** Analyses of the number and sequence of *Polemonium brandegeei* plants visited by foraging *Hyles gallii*. The position of plants in visit sequence, visits per plant, and number of flowers visited per plant were square-root transformed prior to analysis for slope estimates *b* (SE). Initial models included all floral measurements and nonsignificant covariates were deleted using backwards elimination.

Effect	Position in visit sequence	Visits per plant	Number of flowers visited per plant
Array	$F_{6,87} = 2.21$	$F_{5,87} = 1.94$	$F_{5,87} = 2.27$
Herkogamy	n.s.	$F_{1,87} = 28.28^{***}$	$F_{1,87} = 24.70^{***}$
<i>b</i> (SE)		-0.141 (0.027)	-0.134 (0.034)
Mass	$F_{6,87} = 5.31^{**}$	n.s.	n.s.
<i>b</i> (SE)	-1.231 (0.391)		
$R^2$ of model	0.29	0.20	0.24

\*P < 0.050; \*\*P < 0.001; \*\*\*P < 0.0001.



**Figure 3.** The effect of flower mass on the average position of plants within hawkmoth visit sequences. Plants with lower numbers were visited earlier in a foraging bout. Datapoints are adjusted to account for the effects of differences among replicate arrays. Solid line is the predicted sequence position.

even though variation in nectar reward was higher than for any floral dimension other than herkogamy (Appendix A). In addition, nectar volume and sugar content of experimental and field plants were similar (nectar volume mean [SE]: array plants: 2.66 (0.18)  $\mu$ l, field plants: 2.94 (0.24)  $\mu$ l;  $t_9 = 3.6$ , P > 0.05; nectar sugar: array plants: 54.13 (2.94) mg; field plants: 42.86 (3.14) mg;  $t_9 = 3.4$ , P > 0.05). Finally, estimated handling time per flower for each foraging bout did not vary with average floral reward or the weighted average of any other floral traits.

Stigma–anther separation (herkogamy) was the sole variable influencing the number of times a plant was visited and the number of flowers visited per plant (Table 1). Plants displaying recessed stigmas received more visits from hawkmoths than did plants displaying exserted stigmas (Fig. 4A). This result was contrary to our predictions because we expected hawkmoths to respond to floral reward but not to floral dimensions, with the possible exception of flower size.

# FEMALE FUNCTION—POLLEN DEPOSITION AND SEED SET

Pollen deposition varied with both floral traits and hawkmoth behavior. The number of pollen grains deposited on stigmas was positively related to the number of visits received by a plant, and to the length of the corolla tube (Table 2). Pollen deposition was also higher in plants with reverse herkogamy and lower in plants with approach herkogamy (Table 2; Fig. 4B). These independent effects of pollinator preference and floral dimensions on pollen movement were consistent with our expectations.

Seed set (female fitness) varied slightly across arrays and was influenced by herkogamy but not by any other traits (Table 2).



**Figure 4.** Effect of stigma–anther separation (herkogamy) on the number of times a plant was visited by *Hyles gallii* (A), number of pollen grains deposited on the stigmas of *P. brandegeei* array plants (B), and the number of seeds set (C) by array plants after hawkmoth pollination. All points are adjusted to account for the effects of other significant covariates and differences among replicate arrays. Solid lines are the predicted functions for each relationship.

**Table 2.** Analyses of traits affecting female function (pollen deposition and seed set) in experimental populations of *Polemonium brandegeei* after pollination by *Hyles gallii*. Slope estimates, *b* (SE), are in bold and are from untransformed analyses. Selection gradients,  $\beta$  (SE), are in normal typeface and are based on untransformed, but standardized data. Initial models included all floral measurements and visits per plant. Nonsignificant covariates were deleted using backwards elimination.

Effect	Pollen deposition	Seeds set
Array	$F_{5,87} = 1.71$	$F_{5,87} = 5.33^*$
Tube length	$F_{1,87} = 17.85^{***}$	
<i>b</i> (SE)	2.61 (0.621)	n.s.
β (SE)	0.095 (0.02)	
Herkogamy	$F_{1,87} = 6.62^{**}$	$F_{1,87}=5.38^{**}$
<i>b</i> (SE)	-0.040 (0.017)	-0.782 (0.52)
β (SE)	-0.070 (0.030)	-1.05(0.43)
Number of	$F_{1,87} = 6.22^{**}$	$F_{1,87} = 3.33^{1}$
plant visited		
<i>b</i> (SE)	0.139 (0.056)	0.509 (0.29)
β (SE)	0.034 (0.015)	0.445 (0.24)
$R^2$ of model	0.52	0.27

\*P < 0.05; \*\*P < 0.001; \*\*\*P < 0.0001. \* $P \approx 0.078$ .

The effect of array may reflect differences in the number behavior of moths among arrays. As was the case for visits per plant and pollen deposition, seed set was highest in plants with reverse herkogamy and lowest in plants with approach herkogamy (Fig. 4C), resulting in a very strong negative selection gradient  $(\beta = -1.05)$ . Selection on herkogamy through female function was stronger than through male function (Welch's t = -1.14, P < 0.0000.05; Appendix C). The effect of herkogamy was in line with our predictions, but we also expected visits per plant and tube length to affect seed set. The effect of tube length did not approach significance ( $F_{1.87} = 0.05, P > 0.80$ ). However, visits per plant had a marginally nonsignificant effect on seed set ( $F_{1.87} = 3.33, P >$ 0.07), indicating that pollinator preference may have effects on seed set that are independent of sex-organ position. Power analysis with G\*Power 3.1 (Faul et al. 2009) indicated strong statistical power to detect selection through female function (power: 0.96). Although seed set was correlated with total pollen deposition (r = 0.604, P = 0.0028), pollen deposition was not significant in the full ANCOVA model analyzing seed set ( $F_{1,86} = 1.31$ ; P = 0.256).

# MALE FUNCTION—POLLEN REMOVAL AND SIRING SUCCESS

Pollen removal by hawkmoths was influenced only by the number of times a plant was visited (Table 3). Plants receiving more visits

**Table 3.** Analyses of traits affecting male function (pollen remaining in anthers; general linear model and seeds sired; generalized linear model) in experimental populations of *Polemonium brandegeei*, after pollination by *Hyles gallii*. Slope estimates, *b* (SE), are in bold and are from transformed data, whereas selection gradients,  $\beta$  (SE), are in normal typeface and are from untransformed but standardized analyses. Initial models included all floral measurements and visits per plant. Nonsignificant covariates were deleted using backwards elimination.

Effect	Pollen remaining	Seeds sired
Array	$F_{5,87} = 1.46$	$\chi_{1,86}=2.34$
Number of plant visits	$F_{1,87} = 7.21^{**}$	$\chi_{1,86}=3.48^{1}$
<i>b</i> (SE)	-76.79 (28.56)	53.99 (27.4)
β (SE)	-0.034 (0.01)	0.399 (0.31)
Tube diameter		$\chi_{1,86}=4.26^*$
<i>b</i> (SE)	n.s.	-0.345 (0.29)
β (SE)		-0.260 (0.12)
Total sugar content		$\chi_{1,86} = 4.57^*$
<i>b</i> (SE)	n.s.	0.633 (0.25)
β (SE)		0.378 (0.17)
$R^2$ of model	0.21	N/A

\*P < 0.05; \*\*P < 0.01.

 $^{1}P \approx 0.09.$ 

had less pollen remaining in their anthers, and thus more pollen removed, than did plants receiving fewer visits.

We genetically screened a total of 355 offspring, and were able to successfully assign paternity for 275 (77%) at the minimum confidence level of 0.80. The remaining offspring were not assigned paternal components, and therefore removed from the dataset. We assigned paternity to an average of 3.5 offspring per maternal plant.

The number of seeds sired (male fitness) depended on nectar sugar and corolla tube diameter (Table 3; Fig. 5A and B). Strong selection favored higher sugar content ( $\beta = 0.378$ ), whereas weaker but significant selection favored narrow tubes ( $\beta = -0.260$ ). The number of plant visits only had a marginally nonsignificant positive influence on siring success (Wald's  $\chi^2 =$ 3.48, P = 0.099), which contrasted with the strong effect of visit number on pollen removal (Table 3). Finally, the amount of pollen remaining in the anthers was not a good predictor of the number of seeds sired (r = 0.0003; P = 0.998). We had moderate statistical power to detect selection through male function (power: 0.68) as determined with G\*Power 3.1 (Faul et al. 2009).

#### **PROJECTED PURSUIT REGRESSION**

The projected pursuit regressions largely supported our multiple regression analyses. First, analyses of female, male, and total fitness all described primarily linear fitness functions with two effective parameters (mean parameter value for the three



**Figure 5.** Effect of nectar sugar content (A) and corolla tube diameter (B) on male fitness (seeds sired) of *P. brandegeei* array plants after pollination by *Hyles gallii*. All points are adjusted to account for the effects of other significant covariates and differences among replicate arrays. Solid lines are the predicted functions.

analyses = 2.002). Second, the floral traits identified as important for female and male fitness from our ANCOVA's approach also had a large influence on fitness through projected pursuit regression. Herkogamy contributed the most to variation in female function whereas tube diameter contributed the most to variation in male function (Fig. 6). However, the coefficient for nectar sugar content along projection 1 was not statistically significant (Fig. 6). Interestingly, herkogamy was the only variable that contributed significantly to variation in total fitness. Two individuals were removed that had abnormally high male fitness (15 and 18 seeds sired), relative to the rest of our observations. The extreme values of these individuals compressed the other datapoints; this increased the apparent linearity of the spline curve. As we were testing for multivariate selection, we did not want to bias the test. Removal of these two points also improved the GCV score from 0.84 to 0.51 indicating a better fit for the spline curve. However, the removal of these points did not qualitatively change the results of the multivariate analysis.



**Figure 6.** Relative total fitness (A), female fitness (B), and male fitness (C) along the first fitted projection from projected pursuit regression analysis. Solid lines represent the mean bootstrap predictive line, and dashed lines represent a 95% confidence interval. Smoothing parameters ( $\lambda$ ) values were 6, 6, and 10 for A, B, and C, respectively. Values were chosen based on minimized GCV scores. Values below each panel represent mean bootstrap estimates of variable (floral trait) coefficients. Significance of estimates and confidence intervals were generated from 1000 bootstraps.

### Discussion

The attraction of pollinators and facilitation of efficient pollen transfer are important components of reproductive success in flowering plants. We measured these components under a single pollinator in a controlled setting, using plants with a constant flower number. This approach gave us the statistical power to examine in detail how hawkmoths select on floral design, and represents one aspect of selection that would occur in natural populations.

We detected selection on floral design through both female and male function. Nectar sugar and corolla tube diameter were targets of selection through male function, and relative sexorgan position (herkogamy) was selected through female function. Further, selection through female function on herkogamy was stronger than selection through male function on tube diameter (t = -1.56, P < 0.05) but not nectar sugar (t = 4.04, P > 0.05). Plants with recessed stigmas (reverse herkogamy) received more visits, had more pollen deposited, and set more seeds than plants with approach herkogamy. The importance of female function contrasts with predictions in the literature that reverse herkogamy promotes siring success (Webb and Lloyd 1986; Barrett et al. 2000) and the expectation that selection on male function should be stronger than selection on female function (Bateman 1948). Our results suggest that recessed stigmas may promote both pollinator attraction and efficient receipt of pollen.

#### HAWKMOTH PREFERENCES AND FORAGING BEHAVIOR

The preference of hawkmoths for flowers displaying reverse herkogamy was unexpected and, to our knowledge, has not been previously described. Several studies have noted the hovering habit of hawkmoth pollinators; moths remain suspended above flowers, and extend their proboscis to probe flowers (Eisikowitch and Galil 1971; Willmott and Búrquez 1996). Therefore, it may be more efficient for a hawkmoth to forage on flowers with fewer exserted sex organs. The recessed stigmas of reverse herkogamous flowers would not obstruct the foraging target (opening of corolla tube) as much as the exserted stigmas of approach herkogamous flowers. The preference for reverse herkogamy was evident when flower number (floral display) was held constant; additional studies that vary flower number would be required to assess the relative influence of sex-organ position and display size.

Pollinators often prefer larger flowers over smaller ones because information from larger flowers may be more easily processed by insects (Spaethe et al. 2001). Also, large flowers typically offer more floral rewards (Cresswell and Galen 1991; Cresswell 1998; Vaughton and Ramsey 1998), and may therefore provide a visual cue of reward level (Bell 1985; Armbruster et al. 2005). Indeed, hawkmoths have been shown to directly respond to increased reward availability and display (Hodges 1995). We observed an initial preference for large flowers in the sequence of plants visited by *H. gallii*. However, we did not observe a correlation between flower size and reward. Therefore, hawkmoths initially cued in on large flowers but, without an associated reward, the initial preference did not translate into a higher total number of visits to plants with large flowers. In our experiment, higher sugar content did enhance siring success and may have increased the handling time of flowers by hawkmoths. However, we did not observe greater removal of pollen from plants with higher nectar sugar. Nor did we find the average phenotype of plants visited within a foraging bout to be a good predictor of the average time spent visiting a flower. Timed visitation trials with naive and experienced hawkmoths would be required to conclusively determine all aspects of hawkmoth foraging preferences.

Overall, captive hawkmoths in our study appeared to behave similarly to hawkmoths in natural settings. Reports on the number of flowers and plants visited by hawkmoths are rare, but the patterns we observed (13.36  $\pm$  1.49 flowers per bout; Appendix A) were similar to field observations (10.2  $\pm$  2.2 flowers per bout; Kulbaba and Worley, 2008). On average, H. gallii visited a similar number of P. brandegeei flowers per foraging bout as did its close relative H. lineata (11-17 flowers per bout) in natural populations of Ipomopsis tenuituba (Aldridge and Campbell 2007). Hawkmoths foraging in natural populations with more plants may be able to show stronger discrimination among phenotypes than we observed. However, in our study, moths never visited more than half of the available flowers in a bout and seed set appeared to be pollen limited. Therefore, hawmoths were apparently not compelled to visit unattractive phenotypes in our arrays. In our artificial P. brandegeei populations, hawkmoths did not revisit the same plant within a bout. This result implies that hawkmoths retain information about which plants they have recently visited, possibly to avoid flowers already emptied of nectar.

The foraging behavior of pollinators can strongly influence both female and male fitness through effects on outcrossed and self-pollen movement. Visits to multiple flowers on a plant introduce the potential for geitonogamous (self) pollen transfer. Self-pollen deposition under bee pollination is minimized when female-phase flowers are positioned below male-phase flowers because bees typically forage from bottom to top and therefore visit female flowers first (Harder et al. 2000, 2004). In our study, *H. gallii* were equally likely to move upwards or downwards. If this unpredictability is a general feature of hawkmoth pollination, the spatial distribution of floral gender may not affect mating patterns in hawkmoth-pollinated species.

#### SELECTION ON FLORAL DESIGN

Based on the literature, we hypothesized that sex-organ position would affect the efficiency of pollen dispersal under hawkmoth pollination. Specifically, we expected stigmas recessed within narrow corolla tubes (reverse herkogamy) to receive more outcrossed pollen than exserted stigmas. Although recessed stigmas sometimes receive more self-pollen than exserted stigmas under moth pollination (Murcia 1990), the effects of herkogamy on pollen deposition and seed set were very similar in self-sterile *P. brandegeei*. This similarity implies that the larger pollen loads received by reverse herkogamous plants reflected an increase in outcrossed pollen. In contrast to herkogamy, increased tube length was associated with higher pollen deposition but had no effect on seed set. Thus, longer floral tubes may increase self-pollen deposition in *P. brandegeei*, as occurs in *Ipomea trichocarpa* when visited by the moth *Enyo lugubris* (Murcia 1990).

Our analyses also suggested that herkogamy had direct effects on pollen deposition and seed set that were independent of pollinator attraction. Galen and Newport (1987) hypothesized that selection promoting pollination efficiency should be stronger than selection for increased floral rewards, which are energetically expensive, and require additional energy expenditure to increase pollen receipt. Selection on herkogamy was very strong in our experiment and we further suggest that selection on traits that enhance both pollination efficiency and attraction may be stronger than selection on traits that enhance either function alone. Sexorgan position in moth-pollinated plants may be one such trait. A full decoupling of attraction and efficiency would require analysis of pollen loads following single visits by hawkmoths to approach and reverse herkogamous plants. One could then determine if the increased pollen deposition on reverse herkogamous flowers is due to a functional association between recessed styles and hawkmoth morphology or repeated visitation (attraction). Additional measurements would allow us also to explore the possibility that correlations with other traits such as ovule number or pollen viability may contribute to the correlation between herkogamy and seed set.

Selection on sex-organ position may depend on the size and shape of the corolla. A recessed stigma is most efficient when the corolla tube is shorter than the moth's proboscis, as in *P. brandegeei*, so that the proboscis is the pollen-bearing surface (Nilsson, 1988). Although pollen may be deposited toward the mouth, coiling and uncoiling of the proboscis as the moth moves between plants redistributes the pollen along the length of the proboscis (M. Kulbaba, pers. obs.). By contrast, some hawkmoth-pollinated species such as Gladiolus longicollis produce a corolla tube longer than the pollinators' proboscis (Alexandersson and Johnson 2002). These longer tubes force the moths' head deeper into the opening of the flower; thus, pollen is transported on the head instead of the proboscis. This situation may explain why stabilizing selection for approach herkogamy occurs in hawkmoth-pollinated I. wolcottiana (Parra-Tabla and Bullock 2005).

Narrow corolla tubes are generally associated with pollination by lepidopterans (Grant 1983, 1985). Narrow tubes may enhance pollen pickup by increasing the proximity, and likelihood of contact, between the anthers and pollen-carrying proboscis. To our knowledge, our study was the first to demonstrate selection on tube diameter through siring success (male fitness). However, Campbell et al. (1997) detected hawkmoth-mediated selection for narrow corolla tubes through female fitness.

Although selection by hawkmoths may promote reverse herkogamy and narrow corolla tubes, approach herkogamy and relatively wide corollas are common in natural populations of P. brandegeei. An additional and more frequent pollinator of P. brandegeei is the hummingbird S. platycercus (Kulbaba and Worley 2008). Selection through male function for wider corolla tubes occurs in hummingbird-pollinated I. aggregata (Campbell et al. 1991, 1996), whereas selection through female function promotes exserted stigmas (Campbell 1989). Wider corolla tubes and exserted stigmas allowed the pollen-bearing faces of hummingbirds to better remove and deposit pollen, respectively (Campbell 1989; Campbell et al. 1996). Therefore, when selection by only hawkmoths was measured in our study, it is not surprising that we found negative directional selection on corolla width and herkogamy. An analogous experiment to measure hummingbirdmediated selection on P. brandegeei flowers is underway, and should clarify the extent to which different pollinators impose contrasting selection on corolla shape and sex-organ position.

In addition to hawkmoths and hummingbirds, P. brandegeei is frequented by a diverse array of apparently ineffective floral visitors (Kulbaba and Worley 2008). Pollen theft by less-effective visitors could reduce reproductive output by reducing the total available pollen for dispersal (Hargreaves et al. 2010). In other species, interactions with herbivores (Kessler et al. 2011) and resource variation among microsites also influence selection by pollinators (Sánchez-Lafuente et al. 2005). Indeed, environmentally induced variation may often be lower in laboratory than in natural populations (Endler 1986). Thus, the range and distribution of traits we used may have differed from those in natural populations, although mean values were kept similar. All the above factors may moderate the strong selection by hawkmoths that we observed, which exceeded that normally seen in natural populations (Kingsolver and Pfennig 2007). However, our observed selection gradients are consistent with the prediction that hawkmoths maintain reverse herkogamy in natural populations of P. brandegeei.

Natural selection may promote the co-occurrence of particular combinations of traits, without affecting the distribution of either trait individually (Brodie 1992). This possibility is best explored through multivariate analyses of fitness surfaces (Schluter and Nychka 1994; Maad 2000). However, in this study, projected pursuit regression indicated linear selection by *H. gallii*  on *P. brandegeei* floral traits. The results largely confirmed our analyses of covariance, although the effect of nectar sugar content on siring success was not detected through the projected pursuit regression (P > 0.05). As in the ANCOVA, herkogamy had the largest influence on female and total fitness.

#### POLLINATOR RESPONSE, FITNESS SURROGATES, AND GENDER-SPECIFIC SELECTION

Given the logistics of estimating siring success, many studies of natural populations must rely on surrogate measures to estimate male fitness. Surrogates include pollen removal (Maad and Alexandersson 2004), insect visitation rates (Conner and Rush 1996), and pollen dyes (Van Rossum et al. 2010). However, these surrogates sometimes yield biased estimates of male function (Klinkhamer et al. 1994). In our study, reliance on visitation rates or pollen removal would have given an incomplete picture of selection on flower traits through male function. First, selection on tube diameter and sugar content was not apparent in the surrogate measure of male fitness. Second, the removal of pollen by H. gallii was largely influenced by the number of times a plant was visited, as has been found for many other floral visitors including bees (Galen 1989; Galen 1992), birds (Engel and Irwin 2003), and bats (Arias-Coyotl et al. 2006). However, in our study, visit number only had a marginal effect on siring success. Independence of pollen removal and siring success may commonly occur in species producing granular pollen due to pollen wastage, and loss to pollen consumers (Galen 1992; Kobayashi et al. 1999; but see Ashman 1998).

Direct measurements of female fitness are easy to obtain compared to measures of male fitness, although surrogate measures of female fitness are sometimes necessary in communitywide studies (Conner 2006; Alacórn 2010). In our study, pollinator behavior and pollen deposition were reliable indicators of selection on herkogamy through female fitness (Tables 1 and 3). However, longer corolla tubes increased pollen deposition, but did not increase seed set.

The relative direction and magnitude of selection on floral traits through female and male function is still contentious (Sahli and Conner 2011). Selection through male function should be strongest when seed production is limited by available resources (resource limitation) and siring success is limited by mating opportunities (Bateman 1948). However, the magnitude of selection through female function should increase when seed production is limited by mating opportunities (pollen limitation; Wilson et al. 1994; review in Harder and Aizen 2010). Pollen limitation may be common in natural populations (Ashman and Morgan 2004). In our study, selection through female function on herkogamy was much stronger than observed through male function on corolla width and nectar sugar content, perhaps because greenhouse conditions and limited numbers of pollinators resulted in pollen limit-

tation of seed set. Most of the few studies that examined selection through both gender functions have found no major discrepancies in selection strength (O'Connell and Johnston, 1998; Ashman and Morgan 2004; Sahli and Conner 2011; but see Hodgins and Barrett 2008b), nor have many instances of contrasting selection among gender functions been reported (but see <u>Campbell 1989</u>). The discrepancy in the strength of selection between female and male function found in our study highlights the importance of female function in the evolution of floral design.

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## Appendix A

Summary statistics of measured floral traits pooled across all six replicate arrays (n = 94). All measurements are in millimeters except mass (g), sugar (mg) nectar volume ( $\mu$ I), and number of visits (counts). As herkogamy contained negative values, we added the absolute value of the most negative value of herkogamy (3.89) to each value of herkogamy to shift the distribution to the right of zero.

Trait	Minimum	Maximum	Mean	SE	CV
Flower width	9.82	22.14	16.09	0.32	13.8
Tube length	17.13	30.52	22.98	0.34	14.2
Basal tube diameter	1.74	3.98	2.56	0.04	16.63
Apical tube diameter	3.05	5.87	4.03	0.06	14.54
Herkogamy	-3.89	4.62	0.58	0.24	52.89
Style length	19.19	36.67	26.26	0.34	12.70
Anther height	18.28	36.8	25.70	0.39	15.08
Mass	0.003	0.015	0.008	0.0002	27.21
Nectar volume	0.25	7.79	2.66	0.18	64.26
Sugar	1.48	143.40	54.13	2.94	52.58
Flowers visited/Bout <sup>1</sup>	2.00	26.00	13.39	0.49	38.98
Plants visited/bout <sup>1</sup>	1.00	7.00	3.67	0.11	29.47
Foraging time (seconds) <sup>1</sup>	7	196	108.2	7.10	46.84
Avg. flowers visited/plant/bout <sup>1</sup>	1.3	4.5	3.4	0.097	20.68
Total flowers visited/array <sup>2</sup>	112	235	194.17	18.87	9.72
Total visits <sup>1</sup> received by plants	5.0	22.0	13.09	0.43	32.07

<sup>1</sup>Based on n = 51 observed foraging bouts across six replicate arrays.

<sup>2</sup>Based on n = 1165 observed flower visits across six replicate arrays.

## Appendix B

Pearson's correlations of floral traits and the number of flowers visited. Values are pooled across all six replicate arrays. All values were log<sub>10</sub> transformed for normality assumptions except for the number of flower visits, which was square-root transformed. Total flowers visited and total plants visited refer to the number of flower and plant visits documented over each 48-h array period.

	FW	TL	TS	TF	Style length	Anther height	Herkogamy	Nectar volume	Sugar	Mass	Total flowers visited	Total plants visited
FW		0.621	0.443	0.416	0.618	0.569	-0.111	0.001	0.111	0.666	0.126	0.152
TL			0.379	0.491	0.754	0.939	-0.410	0.209	-0.152	0.702	0.244	0.193
TS				0.715	0.437	0.400	-0.086	0.048	0.008	0.681	0.002	-0.025
TF					0.491	0.506	-0.151	0.041	0.014	0.693	0.061	0.004
Style length						0.685	0.145	0.145	0.007	0.684	-0.075	0.005
Anther height							-0.547	0.169	-0.138	0.646	0.282	0.215
Herkogamy								-0.122	0.081	-0.126	-0.463	-0.294
Nectar volume									0.073	0.175	0.107	0.052
Sugar										0.107	-0.070	0.050
Mass											0.072	0.044
Total flower												0.791
visits												

Boldface correlations are significant at P < 0.05.

## Appendix C

Analyses of selection via female (seed set) and male (seeds sired) fitness in experimental populations of *Polemonium brandegeei* after pollination by *Hyles gallii*. Slope estimates, *b* (SE), are in bold and are from transformed analyses. Selection gradients,  $\beta$  (SE), are in normal typeface and are based on untransformed, but standardized data.

Effect	Male fitness (seeds set)	Male fitness
		(seeds sired)
Array	$F_{5,84} = 5.71^{**}$	$\chi_{1,86} = 2.14$
Tube diameter <sup>1</sup>		
<i>b</i> (SE)	-0.040 (0.484)	-0.327 (0.149)*
β (SE)	-0.017 (0.24)	-0.250 (0.12)*
Herkogamy <sup>1</sup>		
<i>b</i> (SE)	-5.703 (1.56)*	-0.021 (0.026)
β (SE)	-1.07 (0.45)*	-0.211 (0.60)
Sugar content <sup>1</sup>		
<i>b</i> (SE)	0.110 (0.181)	0.005 (0.002)*
β (SE)	0.028 (0.15)	0.412 (0.19)*
Number of plant		
visits		
<i>b</i> (SE)	0.163 (0.095)	0.064 (0.013)
β (SE)	0.438 (0.25)	0.384 (0.31)

\*P < 0.05; \*\*P < 0.001.

<sup>1</sup>Indicates significant difference (Welch's t-test; P < 0.05) in the magnitude of  $\beta$  determined through female and male function.