



SELECTION ON *POLEMONIUM BRANDEGEEI* (POLEMONIACEAE) FLOWERS UNDER HUMMINGBIRD POLLINATION: IN OPPOSITION, PARALLEL, OR INDEPENDENT OF SELECTION BY HAWKMOTHS?

Mason W. Kulbaba^{1,2} and Anne C. Worley¹

¹Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

²E-mail: umkulbam@cc.umanitoba.ca

Received August 4, 2012

Accepted February 14, 2013

Data Archived: Dryad doi: 10.5061/dryad.5f1t0

Particular floral phenotypes are often associated with specific groups of pollinators. However, flowering plants are often visited, and may be effectively pollinated by more than one type of animal. Therefore, a major outstanding question in floral biology asks: what is the nature of selection on floral traits when pollinators are diverse? This study examined how hummingbirds selected on the floral traits of *Polemonium brandegeei*, a species pollinated by both hummingbirds and hawkmoths. In array populations of *P. brandegeei*, we measured pollen movement, and female (seeds set) and male (seeds sired) fitness under hummingbird pollination. We then compared the patterns of selection by hummingbirds with our previous study examining selection by hawkmoths. We documented contrasting selection on sex organ positioning through female function, with hummingbirds selecting for stigmas exerted beyond the anthers and hawkmoths selecting for stigmas recessed below the anthers. Furthermore, hummingbirds selected for longer and wider corolla tubes, and hawkmoths selected for narrower corolla tubes. Therefore, contrasting selection by hawkmoths and hummingbirds may account for variation in sex organ arrangements and corolla dimensions in *P. brandegeei*. We documented how floral traits under selection by multiple pollinators can result in either an intermediate “compromise” between selective pressures (sex organs) or apparent specialization (corolla tube length) to one pollinator.

KEY WORDS: Floral selection, herkogamy, hummingbirds, male fitness, pollen movement, selection gradients.

Floral designs are complex phenotypes composed of multiple traits that cumulatively influence interactions with pollinators. Traditionally, individual floral traits have largely been viewed as a response to selection by a single pollinator, or functional group of pollinators (Stebbins 1970). However, many plant species are pollinated by two or more functional groups that may each contribute to selection on floral traits (Waser et al. 1996; Ollerton 2006). The response to selection by multiple groups of pollinators can vary, depending on the strength and nature of selection by each pollinator (Aigner 2001; Sahli and Conner 2011). Therefore,

characterizing selection by each functional group of pollinator is an essential first step toward understanding net selection exerted on floral traits, and thus the evolution of floral design. There exist few independent estimates of selection on floral traits by the diverse pollinators of a single plant species. Even fewer studies have described both female and male fitness under multiple pollinators (but see Sahli and Conner 2011).

Aigner (2001) developed optimality models that predicted how floral traits could respond to a single versus multiple pollinators. Generally, the response of floral traits to selection by

multiple pollinators depended on the strength and targets of selection, and could involve trade-offs in fitness (Aigner 2001, 2004). A fitness trade-off may occur when an increase in fitness under one pollinator corresponds to a decrease in fitness under another pollinator (e.g., Muchhala 2007). More recently, Sahli and Conner (2011) described four potential responses of floral traits to variable selection by two distinct pollinators. First, if pollinators exert contrasting selection on a trait, the mean trait value may be an “intermediate” or “compromise” phenotype between the optimum of each of the two pollinators. Second, if pollinators select similarly on the same trait, the mean trait value may represent an optimum for both pollinators. Third, if selection on a floral trait is much stronger from one of the two pollinators, the mean value of a floral trait may represent a specialization towards that pollinator. This response may occur when a particular trait is more critical for pollination to one pollinator, or the trait optimum for the second pollinator covers a wider range of phenotypes. Finally, the simultaneous presence of both pollinators may change the nature of selection. Therefore, selection may be nonadditive such that the impact on the floral trait cannot be predicted by selection from each pollinator acting singly. These four outcomes may differ among floral traits so that flowers may have a combination of intermediate phenotypes and / or a mosaic of phenotypes apparently specialized to different pollinators (cf. Aigner 2001). The surprisingly few studies that have empirically examined selection on a single species by multiple pollinators found instances of the first three potential outcomes (Castellanos et al. 2004; Muchhala 2007; Sahli and Conner 2011). However, most of these studies examined selection on artificial flowers, or on a small number of floral traits (but see Sahli and Conner 2011). Additional studies are needed to develop a general picture of the frequency of each outcome.

Temporal and spatial variation in the abundance of different pollinators can affect both trait means and genetic variation in floral traits (Forrest et al. 2011). For example, variation in the pollinator assemblage of *Calathea ovandensis* contributed to increased variation in corolla length (Schemske and Horvitz 1984). In contrast, parallel selection on a floral trait by multiple pollinators is expected to reduce the genetic variation in that trait. However, the prevalence of parallel versus contrasting selection by distinct pollinators is unknown (Sahli and Conner 2011).

Regardless of pollinator diversity, the response of heritable traits to selection may be constrained by gender conflict or genetic correlations (Roff 1997). First, the majority of flowering plants are hermaphroditic, and can function as both maternal and paternal parents. As a consequence, contrasting selection through female and male functions has been predicted to be common in hermaphrodites (Morgan 1992). However, few studies have compared gender-specific selection on floral traits because male fitness (seeds sired) is difficult to assess and therefore seldom di-

rectly measured, especially in the presence of multiple pollinators. The limited evidence available suggests that conflicting selection among gender functions is rare (Ashman and Morgan 2004; Sahli and Conner 2011). Second, genetic correlations caused by pleiotropy can also constrain the response to selection, irrespective of gender function or differing targets of selection among pollinators (Conner, 2002; Delph et al. 2004).

Hummingbirds and hawkmoths are important pollinators that have influenced the floral design of many North American plant species (Fægri and van der Pijl 1972; Grant 1983, 1985). A common feature of plant taxa pollinated by hummingbirds is the presence of a tubular corolla of similar length to the bird's bill (Grant and Grant 1968). The correspondence between the corolla and bird's bill lengths enforces contact between plant sex organs and the feathered regions of the bird's head where pollen is carried (Lertzman and Gass 1983). The exertion of sex organs beyond the opening of the corolla tube further reinforces contact between sex organs and the hummingbird. In contrast, plants pollinated primarily by hawkmoths often display flowers with narrow corolla tubes (Nilsson 1988; Alexandersson and Johnson 2002; Brunet 2009; Kulbaba and Worley 2012). Furthermore, plants pollinated by hawkmoths may also display stigmas recessed below the anthers. The presentation of a recessed stigma is thought to increase the proximity of the female sex organs to the slender pollen-bearing moth proboscis (Webb and Lloyd 1986; Barrett 2002). Finally, plants pollinated by hummingbirds often produce large volumes of relatively dilute nectar compared to hawkmoth-pollinated plants (Baker 1972). However, the production of dilute nectar may have evolved more as a deterrent to bee visitation and overly vigorous bird visitation, rather than as a bird attractant (Cronk and Ojeda 2008).

We examined selection by two major pollinators of the subalpine perennial *Polemonium brandegeei*. Natural populations of *P. brandegeei* occur along the Rocky Mountain of North America from New Mexico to southern Montana and appear as far east as south western South Dakota (Davidson 1950). The flowers of *P. brandegeei* display traits associated with hummingbird and hawkmoth pollination, and both animals are confirmed pollinators (Kulbaba and Worley 2008). Overall, *P. brandegeei* flowers exhibit tubular corollas of dimensions that are typical of hummingbird-pollinated taxa (Kulbaba and Worley 2008). However, the cream-white coloration of the corolla, and the strong, heavy sweet smell emitted by the flowers are traits associated with hawkmoth pollination (Grant 1983, 1985). The flowers of *P. brandegeei* display a high level of continuous and heritable variation in style length, resulting in a range of approach (stigmas exerted beyond anthers) to reverse (stigmas recessed below anthers) herkogamy (Kulbaba and Worley 2008).

Here we describe selection on floral traits by hummingbirds in experimental populations, hereafter “arrays.” We then

compare these results to our previous study examining selection by hawkmoths on *P. brandegeei* floral design, where we documented selection for reverse herkogamy through female function, and selection for narrow corolla tubes and high nectar sugar concentration through male function (Kulbaba and Worley 2012). Measurement of hummingbird foraging behavior, pollen deposition, and removal, in addition to the number of seeds set and sired by each plant, allowed us to compare various indices of reproductive success.

We explored the following specific hypotheses. (1) We predicted that increased corolla diameter (as a measure of flower size), or nectar reward (volume, sugar concentration), would result in more visits from hummingbirds and / or increased handling time of flowers. In contrast to floral rewards, we did not expect herkogamy or floral dimensions other than corolla diameter to affect hummingbird behavior, but we did expect these traits to influence female and male fitness through their effects on pollen deposition and removal, respectively. In particular, (2) we expected plants displaying flowers with approach herkogamy to receive more outcrossed pollen, and therefore set more seeds. Finally, (3) we predicted that plants displaying flowers with longer corolla tubes would have more pollen removed from their anthers, and sire more seeds than plants with shorter corolla tubes because the corolla tubes of *P. brandegeei* flowers are generally shorter than the hummingbird's bill (Kulbaba and Worley 2008).

Materials and Methods

GENERAL DESIGN

To measure selection by hummingbirds on the floral traits of *P. brandegeei*, we used a similar approach to our previous study that measured selection by hawkmoths (Kulbaba and Worley 2012). We presented arrays of plants to the ruby-throated hummingbird (*Archilochus colubris*), and measured pollen movement (pollen removal and deposition), and selection gradients on floral traits through female (seeds set) and male (seeds sired) fitness. Although, *A. colubris* is not a confirmed pollinator of *P. brandegeei*, it is similar in behavior and morphology (most importantly bill length: 13.4–19.0 mm) to the confirmed hummingbird pollinator, *Selasphorus platycercus* (16.0–20.3 mm; Pyle 2001).

We grew experimental plants from seed collected at Taylor Canyon, Colorado (39°34'33"N 104°22'26"W) under greenhouse conditions in 800 mL conical Deepots® with Premier Pro Mix® medium and Osmocote Plus® slow-release fertilizer (10 : 10: 10). A total of five arrays were used in our experiment. Each array consisted of 12–14 plants (total of 62 individuals) that were chosen based on their floral phenotypes. As one of our major goals was to examine how hummingbirds selected on variable stigma-anther separation (herkogamy), we ensured an equal number of approach and reverse herkogamous plants in each array. Plants

were arrayed in a circular arrangement as indicated in figure 1 of Kulbaba and Worley (2012). Such an arrangement of plants included alternating approach and reverse herkogamous individuals, which provided an even distribution of herkogamous phenotypes and an equal probability of hummingbirds encountering either phenotype upon entering the array. Finally, we standardized all experimental inflorescences to seven freshly opened flowers that remained open for the duration of the experiment. The range of herkogamy and corolla trait variation in our arrays was similar to variation observed in natural populations (Kulbaba and Worley 2008).

We conducted the experiments in two locations in rural Manitoba (approximately 174 km separation), to ensure that different birds were involved in the pollination of experimental plants. The first location was in an oak / aspen woodland north of Anola, Manitoba (49°56.355'N 96°36.734'W). Our second location was west of Overton, Manitoba (51°00.719'N 98°46.664'W), in another oak / aspen clearing. In May 2010, we presented ruby-throated hummingbirds with feeders consisting of 30% sucrose solution, to entice hummingbirds to our study sites. Feeders remained at the sites for the duration of the experimental period, but were emptied and thoroughly washed before arrays were assembled.

Hummingbirds visited each array for a total of two consecutive days (approximately 7:00 a.m. to 8:30 p.m.). We presented the arrays to hummingbirds between 18 July 2010 and 27 August 2010 when weather and the availability of floral phenotypes permitted. The empty and cleaned feeders remained in the center of the arrays, because the hummingbirds had been habituated to their presence. An observer was present for the entire duration of each experiment, to discourage any nonbird visitors from foraging on the arrays. After dusk, the array plants were placed indoors to prevent nocturnal visitors from foraging.

HUMMINGBIRD BEHAVIOR

All hummingbird foraging bouts were recorded with a video camera to document foraging behavior on *P. brandegeei* flowers. For each foraging bout, we calculated the total number of flowers visited during the bout, the total number of plants visited, and the number of flowers visited per plant. We calculated handling time for visits to individual flowers by dividing the number of frames the visit lasted by the video frame rate (29 frames/sec). Mean handling times were calculated for individual plants by averaging the individual handling times of flowers on each array plant. We also examined the sequence of plants visited by hummingbirds, within foraging bouts. Finally, we examined the movement of hummingbirds within an inflorescence by comparing the number of upward versus downward movements within an inflorescence. The pattern of intraplant movement has been shown to affect the segregation of sex functions within inflorescences (Harder et al. 2004).

FLORAL TRAITS, POLLEN MOVEMENT, AND FITNESS

We measured floral dimensions and nectar properties (volume and sugar concentration) before birds visited each array. Two to three freshly opened flowers from each plant were destructively measured for floral traits and nectar properties before each array, while consistently maintaining seven open flowers per plant. Floral trait measurements and nectar properties were averaged for each experimental plant. Measured traits included: corolla width (distance from the tip of one petal to the opposing petal tip), corolla tube length, corolla tube width at the apex and base of the flower, sex organ separation (herkogamy), presentation of sex organs relative to the base of the flower, and flower mass (dry weight of corolla, calyx, androecium, and gynoecium). Herkogamy was measured as the distance between the stigma and the closest anther. Plants with recessed stigmas had a negative value of herkogamy and plants with exerted stigmas had positive values of herkogamy. See figure 2 of Kulbaba and Worley (2012) for precise location of measurements. Nectar sugar concentration was determined with the aid of a calibrated handheld refractometer (Fisher Scientific), with a temperature correction. We corrected our values with the conversion table in [Kearns and Inouye \(1993; Table 52, p. 172\)](#) because small volumes of nectar can introduce error when estimating the concentration of sucrose equivalents. Finally, 11 plants were reused in three replicate arrays, but flowers on different inflorescences were used. Therefore, we resampled the nectar properties to determine if nectar volume or sugar concentration changed, and obtained new floral measurements prior to assembling each array. Furthermore, we performed a repeated measures analysis of variance on the number of seeds set and seeds sired, to explore the consistency of female and male fitness in these plants.

We measured pollen deposited on stigmas and pollen remaining in anthers of *P. brandegeei* plants after pollination by hummingbirds. We allowed 24 h in the greenhouse for fertilization, because a trial experiment with hand-pollinated plants determined this time period to be sufficient for ovule fertilization (Kulbaba and Worley 2012; M. Kulbaba, unpubl. data). Stigmas were removed from five of the experimental flowers and mounted on fuchsin jelly slides to stain pollen grains (Beattie 1971). We quantified pollen deposition by manually counting the pollen grains on the stigma from digital images (Image-pro express; Media Cybernetics Inc., Rockville, MD). As we were unable to distinguish between self and outcross pollen, pollen deposition measures reflected both. All five anthers from five of the experimental flowers were collected and stored in 70% ethanol. The number of pollen grains remaining in anthers after hummingbird visitation was assumed to indicate pollen removal. Pollen remaining in anthers was quantified with a Multisizer 3 particle counter (Beckman-Coulter, Fullerton, CA), and averaged over the five flowers per plant.

Fitness was assessed through direct measures of female, male, and total fitness. Female fitness was the number of seeds set per plant, and male fitness was the number of seeds in an array sired per plant. Mature seeds from array plants were collected, and germinated under the same conditions as the experimental array plants. Offspring were raised to the seedling stage, when leaf tissue was collected and dried in silica gel. Whole DNA from up to five random offspring per individual array plant was extracted from dried leaf tissue, and genetically screened with six polymorphic microsatellite loci as described in Kulbaba and Worley (2011). We manually scored microsatellite profiles with genemapper 4.0 (Applied Biosystems, Foster City, CA), and then analyzed the profiles with the parentage program Cervus v 3.03 ([Kalinowski et al. 2007](#)). Cervus 3.03 determines the most likely paternal parent with a maximum-likelihood approach ([Meagher 1986](#)). Mendelian segregation probabilities were first simulated based on 10,000 cycles, complete sampling of 11–13 individuals (reflecting array size minus the known maternal parent, because *P. brandegeei* is self-sterile). The simulation included 0.750 as the proportion of loci typed, and a typing error of 0.01. We chose a strict confidence level of 0.95, and a relaxed level of 0.80 because we did not have to consider pollen outside the array populations (cf. [Nishizawa et al. 2005; Hodgins and Barrett 2008](#)).

STATISTICAL ANALYSIS

We examined associations between floral traits and (1) pollinator behavior, (2) pollen removal and deposition, and (3) female and male fitness. We used the same general analysis of covariance (ANCOVA; Proc GLM, Proc GENMOD) approach that we used in the hawkmoth selection study (Kulbaba and Worley 2012). Array number and site were included as fixed effects in all analyses. Hummingbird visitation behavior included handling time, number of flowers visited per plant, and the number of times a plant was visited over all foraging bouts (“plant visits”). These variables were each used as dependent variables with floral traits used as explanatory variables (covariates). We assessed female and male function by analyzing each of pollen deposition, pollen removal, number of seeds set and number of seeds sired per plant as dependent variables with floral traits, and variables describing visitation behavior as explanatory variables (covariates). Initial models included all two-way interactions between covariates and fixed effects, as well as two-way interactions among covariates. We removed nonsignificant interactions and covariates in a reverse step-wise manner ([Sokal and Rohlf 1995](#)). All analyses were performed in SAS 9.1.2 (SAS Institute 2004).

Standardized selection gradients (β) were estimated as the coefficient between standardized fitness measures and floral traits. We standardized fitness measures (seeds set and seeds sired) across all arrays by dividing individual fitness measures by mean

fitness (Lande and Arnold 1983). Finally, we calculated total relative fitness as the average of relative female and relative male fitness to prevent larger values of female fitness from overwhelming total fitness. We chose two techniques to standardize our estimates of selection gradients. First, we standardized the traits by dividing individual trait values by the mean trait value calculated over all arrays, such that the mean trait value was 1 (Hereford et al. 2004). We used this approach because we were particularly interested in how pollinators selected on a highly variable floral trait, stigma–anther separation (herkogamy) relative to other less variable traits (e.g., tube diameter). Second, to compare our results to the majority of other studies measuring selection on floral traits, we also employed the variance-standardized method of Lande and Arnold (1983). Trait values were standardized to a mean of 0 and a variance of 1 (Lande and Arnold 1983). Variance-standardized traits were calculated as the difference between individual trait values and trait means, divided by the trait standard deviation across all arrays.

Linear (β) and quadratic (γ) selection gradients were examined for all explanatory floral traits, and were both included in the analyses. Slope estimates (b) are from analyses of transformed data, but selection gradients are reported from analyses of standardized but untransformed data, because estimates from transformed fitness measures can be biased (Lande and Arnold 1983). Finally, we compared the magnitude of selection gradients between female and male fitness, under a given pollinator with a Welch's t -test, assuming unequal variances.

To test for potential multivariate or correlated selection, we analyzed nonlinear multidimensional fitness surfaces. We used the projected pursuit regression approach described in Schluter and Nychka (1994) to fit spline curves to our fitness data without making assumptions about the shape of the fitness function. Projected pursuit regression provides a more powerful technique to detect correlated selection than the multiple regression approach. Furthermore, determining the number of effective parameters of spline curves can objectively determine how linear or nonlinear a given fitness function is. We analyzed fitness surfaces for relative female, male, and total fitness.

Results

Our analyses provided the opportunity to detect both linear and nonlinear selection through analysis of covariance, and multivariate projected pursuit regression. All interaction and quadratic terms in the ANCOVA models were nonsignificant, and removed from the models. Therefore, the effects of covariates in the present analyses were linear, and independent of other covariates. These results were reflected in the projected pursuit regressions. Selection gradients from the two standardization techniques were very similar, with selection gradients from mean standardized traits

Table 1. Analyses of *Archilochus colubris* handling time and the number of flowers visited per plant on arrays of *Polemonium brandegeei*. Handling time was log-transformed and the number of flowers visited per plant was square root transformed before analysis. Slope estimates b (SE) are in bold face. Initial models included all floral measurements and nonsignificant covariates were deleted using backwards elimination.

Effect	Number of flowers visited per plant	Handling time per flower
Array	$F_{4,56}=10.85^{***}$	$F_{4,56}=9.54^{***}$
Nectar volume	$F_{1,56}=6.07^{**}$	$F_{1,56}=3.78^{\dagger}$
b (SE)	−0.337 (0.14)	0.012 (0.01)
R^2 of model	0.47	0.46

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, $^{\dagger}P < 0.075$.

being slightly smaller in magnitude than selection gradients estimated using variance standardized traits. We therefore present selection gradients from mean standardized analyses, as a conservative estimate of selection on floral traits. Furthermore, the effects of different sites (Overton and Anola) did not affect female ($F_{1,52} = 0.28$; $P = 0.599$) or male fitness (Wald's $\chi^2 = 0.19$; $P = 0.665$). Therefore, analyses presented below do not include site effects.

POLLINATOR FORAGING BEHAVIOR

Ruby-throated hummingbirds visited an average of 5.8 plants, 3.3 flowers per plant, and an average total of 17 flowers per foraging bout. Visits to individual flowers were very brief, with a mean handling time of 0.74 sec (range: 0.34–1.33 sec). Although all array plants were visited in each of the replicate arrays, a small fraction of the available plants were visited in each bout. We observed a total of 863 movements between flowers, within an inflorescence. A total of 405 movements occurred in an upward direction, whereas the remaining 458 proceeded in a downward direction; this did not correspond to a significant difference ($\chi^2 = 2.48$ for expectation of equal number of upward and downward movements $P = 0.214$). A summary of arrays and bird behaviors is presented in Appendix S1.

The volume of nectar produced by *P. brandegeei* flowers affected two aspects of hummingbird foraging behavior. First, hummingbirds visited fewer flowers per plant on plants with larger volumes of nectar per flower. Second, hummingbirds spent longer handling flowers with larger volumes of nectar (Table 1), but this relationship was marginally nonsignificant. The effect of array was significant in both analyses, and may have resulted from different numbers of visiting birds and bird behaviors across arrays. Across all arrays, we observed an average of 35.8 foraging bouts, but the number of bouts ranged from 17 (Array 1) to 52 (Array 4; Appendix S1).

Table 2. Analyses of female function (pollen deposition and seed set) in arrays of *Polemonium brandegeei* after pollination by *Archilochus colubris*. Slope estimates, *b* (SE), are in bold and are from log-transformed data. Selection gradients, β (SE), are in italics and are based on untransformed data standardized to a mean of 1 (Hereford et al. 2004). Initial models included all floral measurements and visits per plant. Nonsignificant covariates were removed using backward elimination.

Effect	Pollen deposition	Seeds set
Array	$F_{4,56}=0.17$	$F_{4,56}=9.74^*$
Tube diameter	$F_{1,56}=30.86^{***}$	$F_{1,55}=1.75^\dagger$
<i>b</i> (SE)	1.148 (0.207)	9.891 (5.644)
β (SE)	<i>1.223 (0.816)</i>	<i>0.858 (0.489)</i>
Herkogamy		$F_{1,55}=29.42^{***}$
<i>b</i> (SE)	n.s.	11.029 (1.971)
β (SE)		<i>0.913 (0.171)</i>
R^2 of Model	0.37	0.52

** $P < 0.001$, *** $P < 0.0001$, $^\dagger P = 0.057$.

As we predicted, hummingbirds did not respond to variation in corolla tube dimensions or sex organ positioning. Unexpectedly, the sequence of plants visited by hummingbirds, and the total number of times an individual plant was visited did not vary with corolla diameter or nectar characteristics.

FEMALE FUNCTION—POLLEN DEPOSITION AND SEEDS SET UNDER HUMMINGBIRD POLLINATION

Basal-tube diameter (hereafter tube diameter) affected pollen deposition. Plants displaying flowers with relatively wide tubes had more pollen deposited on their stigmas after hummingbird visitation (Table 2; Fig. 1). The relationship between seed set and corolla-tube diameter was marginally nonsignificant ($P = 0.057$; Table 2). However, the relationship between tube diameter and total fitness was significant (see *Total Fitness* below). In addition, the number of pollen grains deposited on stigmas was significantly correlated with seed set ($r = 0.266$; $P < 0.05$). Finally, the plants that were reused in multiple arrays did not set significantly different numbers of seeds across the arrays, as determined through repeated measures analysis of variance ($F_{2,20} = 0.03$; $P = 0.970$).

Herkogamy strongly influenced female fitness. On average, plants displaying flowers with approach herkogamy and exerted stigmas set more seeds than did plants displaying flowers with reverse herkogamy (Table 2; Fig. 1). This resulted in strong selection ($\beta = 0.913$) for approach herkogamy through female function, which supported our functional predictions of sex organ arrangement under hummingbird pollination. Curiously, we did not detect a significant effect of herkogamy on pollen deposition ($F_{1,52} = 0.94$; $P = 0.337$).

The total number of hummingbird visits per plant and the number of flowers visited per plant did not affect pollen deposition or seed set in our arrays. This result contradicted our expectations, because pollinator visitation patterns typically influence pollen movement patterns.

MALE FUNCTION—POLLEN REMOVAL AND SEEDS Sired UNDER HUMMINGBIRD POLLINATION

Corolla tube length but not hummingbird visitation patterns affected pollen removal in the arrays. Plants displaying flowers with relatively long corolla tubes had less pollen remaining in their anthers, and therefore more pollen removed after hummingbird visits (Table 3; Fig. 2). This result supported our hypothesized relationship between tube length and pollen removal under hummingbird pollination.

We genetically screened 310 offspring across five replicate *P. brandegeei* arrays, and successfully assigned paternity to 272 individuals (88%), at the minimum confidence level of 0.80. We were unable to confidently assign parentage for the remaining 38 offspring, and therefore removed them from subsequent analyses. We therefore assigned paternity for an average of 4.3 out of five offspring per array plant. Furthermore, plants reused in multiple arrays did not sire significantly different numbers of seeds as determined through repeated measures analysis ($F_{2,20} = 0.473$; $P = 0.641$).

We detected selection for longer corolla tubes through the number of seeds sired (Table 3; $\beta = 0.124$), which supported our prediction of a functional relationship between corolla design and male fitness (Fig. 2). Plants that received relatively more hummingbird visits also sired more offspring. Although we detected weak but significant selection on the number of visits to plants through siring success (Table 3; $\beta = 0.069$), the number of hummingbird visits did not affect the amount of pollen remaining in anthers. Finally, more pollen remaining in anthers (decreased pollen removal) translated into fewer seeds sired ($r = -0.568$; $P < 0.0001$) or set ($r = -0.258$; $P < 0.05$).

TOTAL FITNESS AND COMPARISONS BETWEEN GENDERS AND POLLINATORS

Selection gradients estimated under total relative fitness were qualitatively congruent with those estimated under individual gender functions. For example, selection gradients describing total fitness were approximately half of selection gradients determined by female or male fitness measures alone (Table 4). Two exceptions existed. First, the selection gradient for the number of visits to plants under hawkmoth pollination was significant for total fitness, but nonsignificant under each gender function separately. Second, the selection gradient for total fitness for the number of visits to plants under hummingbird pollination was very similar to the selection gradient determined through male fitness.

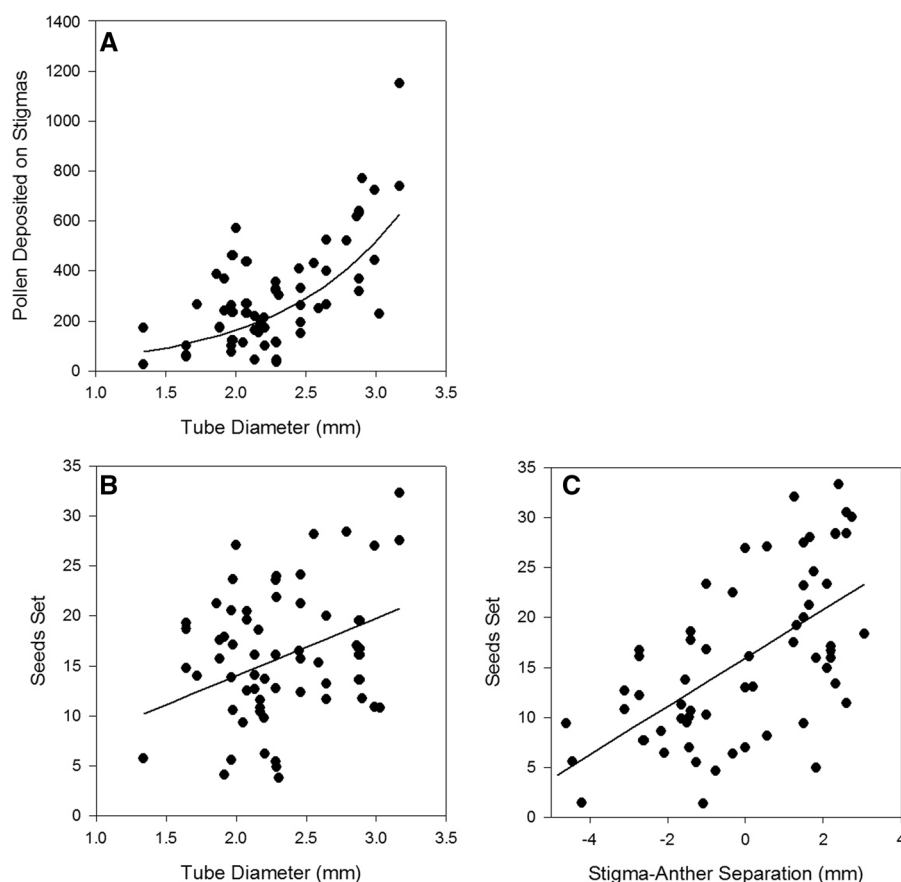


Figure 1. Effects of corolla tube diameter (A and B) and stigma–anther separation (C) on the number of pollen grains deposited on stigmas (A) and number of seeds set (B and C) after pollination by *Archilochus colubris*. Points are adjusted values to account for the effects of other significant variable in the model. See Materials and Methods of for further details.

Finally, selection through female function was stronger than selection through male function. Significant selection gradients on female function were significantly larger in magnitude than significant selection gradients determined through male function based on pair-wise comparisons (all Welch's $t \geq 4.57$; $P \leq 0.045$). The exception was selection on nectar sugar concentration through male function compared to selection for reverse herkogamy under hawkmoth pollination (Welch's $t = 2.87$; $P = 0.102$).

FITNESS SURFACES

Projected pursuit regression indicated that selection by hummingbirds on floral traits was primarily linear. The results generally supported our initial ANCOVA models, especially for female and male fitness. Linear functions (average effective number of parameters 2.004) described relative female, male, and total fitness. Furthermore, the floral traits identified by analysis of covariance were also important in the projected pursuit regression. Specifically, relative female fitness was largely affected by positive selection on herkogamy, and male fitness largely varied with positive selection on corolla tube length. Total relative fitness varied positively with herkogamy and corolla tube diameter (Fig. 3). Tube diameter

was not significant in the multivariate analysis of female fitness but did affect total fitness in the ANCOVA models (Table 4). Finally, flower mass had moderate effects on all three measures of fitness, but was not significant in the ANCOVA models or the projected pursuit regression analysis.

Curiously, significant selection through total fitness was only detected on two traits, corolla tube diameter and herkogamy, whereas our ANCOVA analyses also identified selection on tube length and number of visits per plant. These differences could reflect a positive correlation between the tube dimensions and the fact that we were able to statistically account for differences among arrays in the ANCOVAs but not in the projected pursuit regression.

Discussion

The selection exerted by pollinators on floral traits can have a major influence on floral evolution. We examined selection by hummingbirds on the floral traits of *P. brandegeei* under controlled conditions with a standardized number of flowers. Our approach allowed for a comprehensive description of hummingbird–flower interactions in *P. brandegeei* at the behavioral, pollen movement,

Table 3. Analyses of male function (pollen remaining in anthers and seeds sired) in arrays of *Polemonium brandegeei* after pollination by *Archilochus colubris*. Slope estimates, *b* (SE), are in bold and are from log-transformed data, whereas selection gradients, β (SE), are in italics and are from untransformed data standardized to a mean of 1 (Hereford et al. 2004). Initial models included all floral measurements and visits per plant. Nonsignificant covariates were deleted using backward elimination.

Effect	Pollen removal	Seeds sired
Array	$F_{4,56}=68.53^{**}$	Wald's $\chi^2=4.52^*$
Tube length	$F_{1,56}=111.63^{***}$	Wald's $\chi^2=42.31^{***}$
<i>b</i> (SE)	-0.162 (0.052)	0.126 (0.019)
β (SE)	<i>-0.145 (0.020)</i>	<i>0.124 (0.040)</i>
Plant visits		Wald's $\chi^2=6.57^*$
<i>b</i> (SE)	n.s.	0.301 (0.099)
β (SE)		<i>0.069 (0.009)</i>
R^2 of Model	0.76	—

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

and fitness levels. Finally, this description of selection by hummingbirds allowed us to compare the patterns of selection by birds with a previous study describing selection on *P. brandegeei* floral traits under hawkmoth pollination (Kulbaba and Worley 2012).

This study detected selection by hummingbirds on both the placement of sex organs and corolla dimensions of *P. brandegeei* flowers. Unexpectedly, selection on floral traits through female function was stronger than selection through male function. We detected strong selection on sex organ placement through female function (seeds set), and moderate selection on corolla tube length through male function (seeds sired), with hummingbirds selecting for exerted stigmas and longer corolla tubes. Although we did not detect a conflict in selection on floral traits between gender functions, selection for wider corolla tubes by hummingbirds may constrain the response to selection by hawkmoths favoring narrow tubes. This scenario highlights the need to examine selection by all effective pollinators to understand the evolution of complex floral phenotypes. Our study illustrates how selection by multiple agents could result in intermediate compromises between selective pressures (sex organ position), or a mosaic of apparently specialized floral phenotypes (corolla tube).

HUMMINGBIRD BEHAVIOR AND FORAGING PATTERNS

Variation in *P. brandegeei* floral design did not influence the number of plants or flowers visited by hummingbirds, although we predicted that hummingbirds would prefer wider, more visible flowers. Indeed, selection on morphological traits (e.g., flower size) is often associated with a correlation between a trait and floral rewards (Mitchell 1993). The apparent lack of preference in our study may reflect the lack of a strong indicator of nectar

rewards. Therefore, hummingbirds were unable to associate a visual cue with greater rewards, to develop a preference within *P. brandegeei* flowers.

Hummingbirds did not visit more flowers on plants producing larger floral rewards, or visit these plants more often; this result contradicted our expectation. By contrast, hummingbirds preferred plants of *Penstemon centranthifolium* with larger nectar rewards (Wilson and Jordan 2009). Hummingbirds should have had sufficient time to identify plants with larger rewards in our arrays because hummingbirds are able to identify larger rewards in a very short period of time, and can concentrate their foraging on large rewarding plants (Gass and Sutherland 1985; Brown and Gass 1993; Mitchell 1993). Although the number of times a plant was visited was not related to the floral traits we measured, plants receiving more hummingbird visits sired more seeds but did not set more seeds. Increased rates of visitation are often associated with greater pollen removal and siring success, but not necessarily greater seed set (Stanton et al. 1986; Sahli and Conner 2011). This increased siring success of *P. brandegeei* individuals that received relatively more hummingbird visits supports Bateman's (1948) principle of greater attraction enhancing male function. However, the mechanism(s) influencing hummingbird visitation rates was not clear in our experiment.

Hummingbirds probed flowers for longer when they produced relatively larger volumes of nectar, although this result was marginally nonsignificant. A similar response was detected in hummingbird pollinated *Ipomopsis aggregata*, where longer handling times were required to consume larger volumes of nectar (Mitchell and Waser 1992). However, in our study longer handling times of flowers did not affect pollen transfer, or the number of seeds set or sired.

Hummingbirds visited more flowers per plant when nectar rewards were small. This increase in the number of flowers per plant visited may reflect a short-term response to nectar rewards by hummingbirds, to maximize nectar intake from plants with small rewards. When a foraging hummingbird encounters a plant with smaller nectar volumes, the bird may have to visit multiple flowers to meet the high energetic demands of a hovering pollinator (Baker 1972; Hurly 2003). Alternatively, the foraging behavior observed by hummingbirds in our study may reflect the relatively small number of plants available, and therefore resources available in the arrays. However, this alternative seems unlikely, because the hummingbirds were able to seek other food sources outside the arrays but continually returned to the array plants.

Pollinator movements within inflorescences can have important consequences for the movement of self and outcross pollen within a plant. When multiple flowers per inflorescence are visited, the potential for the movement of self-pollen increases (Harder et al. 2004). Therefore, the segregation of functional genders within an inflorescence (e.g., female phase flowers

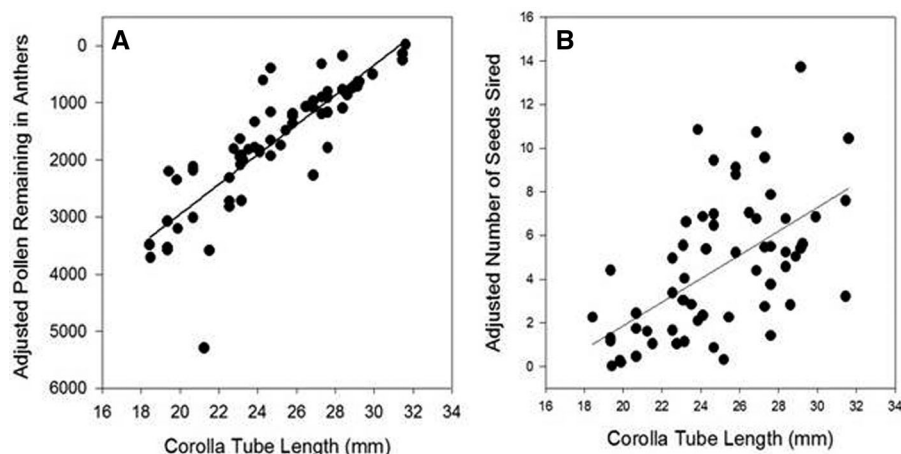


Figure 2. Effect of corolla tube length on pollen remaining in anthers (A) and the number of seeds sired (B) after pollination by *Archilochus colubris*. Points are adjusted values to account for the effects of other significant variable in the model. See Materials and Methods for further details.

Table 4. Selection gradients (standard error) through female (seeds set) and male (seeds sired) function, and summary statistics of fitness measures of *Polemonium brandegeei* under hawkmoth and hummingbird pollination. Selection gradients are from traits standardized to a mean of 1. All significant estimates ($P < 0.05$) appear in bold face.

Effect	Hawkmoth pollination			Hummingbird pollination		
	Female fitness (seeds set)	Male fitness (seeds sired)	Total fitness	Female fitness (seeds set)	Male fitness (seeds sired)	Total fitness
Nectar sugar						
Concentration	$\beta = -0.01$ (0.08) ¹	$\beta = 0.41$ (0.19)¹	$\beta = 0.21$ (0.19)	$\beta = -0.12$ (0.20)	$\beta = 0.01$ (0.34)	$\beta = 0.01$ (0.03)
Tube length	$\beta = 0.01$ (0.10)	$\beta = -0.04$ (0.13)	$\beta = -0.03$ (0.06)	$\beta = 0.19$ (0.61)	$\beta = 0.12$ (0.04)	$\beta = 0.16$ (0.02)
Tube diameter	$\beta = -0.02$ (0.24) ¹	$\beta_1 = -0.26$ (0.12)¹	$\beta = -0.16$ (0.04)	$\beta = 0.86$ (0.49) ^{*1}	$\beta = 0.08$ (0.84) ¹	$\beta = 0.11$ (0.05)
Herkogamy	$\beta = -1.05$ (0.43)¹	$\beta = -0.14$ (0.12) ¹	$\beta = -0.50$ (0.07)	$\beta = 0.91$ (0.17)¹	$\beta = 0.01$ (0.19) ¹	$\beta = 0.28$ (0.14)
Number of visits to plants	$\beta = 0.45$ (0.24)	$\beta_1 = 0.40$ (0.31)	$\beta = 0.16$ (0.08)	$\beta = 0.13$ (0.18)	$\beta = 0.07$ (0.01)	$\beta = 0.05$ (0.02)
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Pollen in anthers	1871.73	232.39	5688.40	1801.95	114.04	8660
Pollen on stigma	485.61	21	1406.0	302.13	25.6	1030
Seeds set	14.63	0	45	11.52	0	35
Seeds sired	2.47	0	7	4.39	1	12

* $P = 0.057$.

¹Indicates significant difference (Welch's t -test; $P < 0.05$) in the magnitude of β when compared across female and male fitness, under a given pollinator.

below male phase flowers) may match the foraging patterns of pollinators within inflorescences (Harder et al. 2000, 2004). For example, bees typically move from lower to higher positions on an inflorescence, reinforcing the spatial separation of genders on an inflorescence. Hummingbirds did not exhibit any difference between the number of upward and downward movements within an inflorescence. Therefore, gender segregation within the inflores-

cence of *P. brandegeei* may not be critical under hummingbird pollination, as has been demonstrated with artificial flowers (Harder et al. 2004).

SELECTION BY HUMMINGBIRDS ON FLORAL DESIGN

We detected directional selection by hummingbirds for wider corolla tubes through female and total fitness, and longer corolla

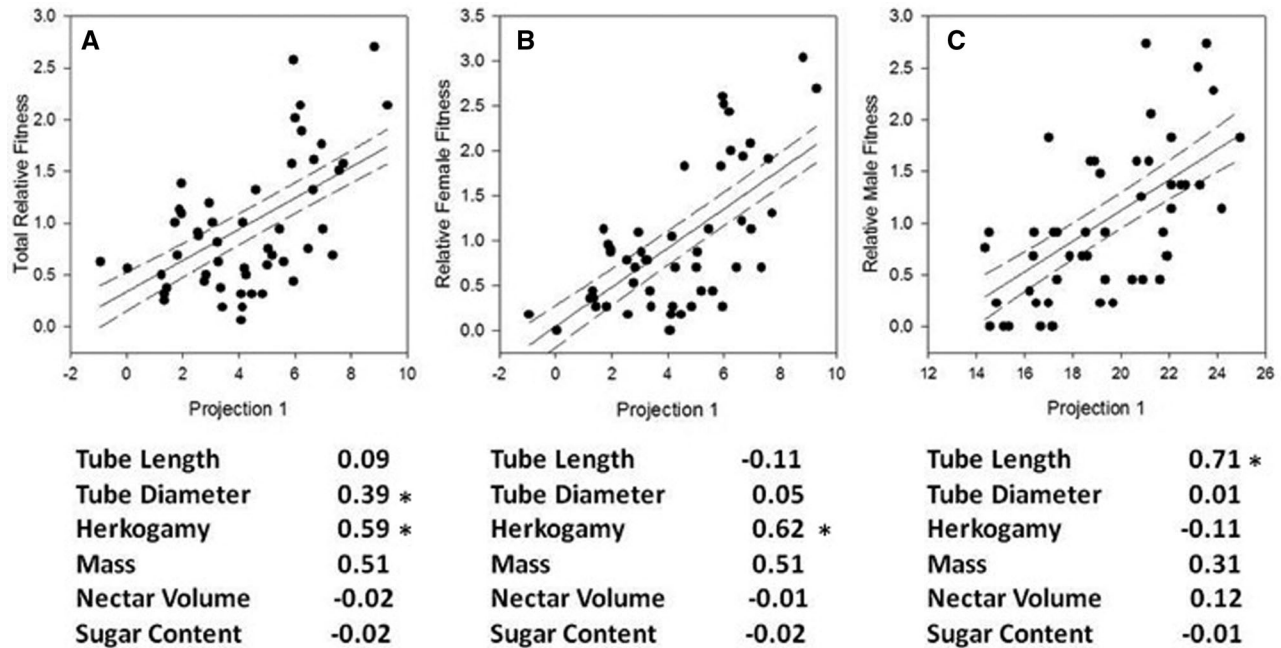


Figure 3. Relative total fitness (A), female fitness (B), and male fitness (C) along the first fitted projection from projected pursuit regression analysis. Solid lines represent the mean bootstrap predictive line, and dashed lines represent a 95% confidence interval. Smoothing parameters (λ) values were 6, 6, and 9 for A, B, and C, respectively. Values were chosen based on minimized generalized cross-validation (GCV) scores. Values below each panel represent mean bootstrap estimates of variable (floral trait) coefficients. Confidence intervals were generated from 1000 bootstraps.

tubes through male fitness. These patterns of selection largely fit with our initial expectations, and other findings in the literature. Although the effect of tube diameter was marginally nonsignificant when measured through female fitness, a significant effect was determined through total fitness (Table 4). Therefore, combining female and male fitness into total fitness may have resolved a lack of power to detect a significant effect of tube diameter on female fitness alone. Similarly, tube diameter affected total fitness but not female or male fitness in the projected pursuit regression. Campbell et al. (1996) detected similar selection through female fitness by hummingbirds for wide corolla tubes in *I. aggregata*. Wider corolla tubes allowed birds to fit more of their pollen-bearing face into a flower, further enforcing contact with sex organs (Temeles 1996). Furthermore, corolla tubes that match or are slightly longer than the hummingbird's bill increase the contact between sex organs, and the pollen-bearing surfaces of hummingbirds (Grant and Grant 1968; Grant and Temeles 1992; Temeles et al. 2009; de Waal 2010). Although our study detected selection for longer corollas through male function and total fitness in the ANCOVA, other studies have found similar selection on corolla length through female function (Campbell 1996; Campbell et al. 1996).

After the initial attraction to and orientation of pollinators at a flower, the relative positioning of sex organs (herkogamy) influences pollen placement. At this stage of the plant–pollinator in-

teraction, the placement of pollen on a region of the animal where it can be deposited on the stigmas of subsequently visited flowers is critical (Webb and Lloyd 1986; Barrett 2002). As predicted, plants in our arrays that displayed stigmas exerted beyond the anthers (approach herkogamy) set more seeds under hummingbird pollination than those displaying stigmas recessed below anthers (reverse herkogamy). Stigmas exerted beyond the opening of the corolla tube make stronger contact with the face and chin regions of hummingbirds, where pollen is carried (Grand and Grant 1968). A similar affect of herkogamy was detected through total fitness as well, but the magnitude was considerably less compared to selection detected through female function alone (Table 4). Other studies have detected selection for exerted stigmas in hummingbird-pollinated taxa through female function (Campbell 1996; Campbell 1996; but see Castellanos et al. 2004). For example, Reynolds et al. (2009) detected consistent long-term selection in 8 out of 9 years for exerted stigmas in *Silene virginica* by ruby-throated hummingbirds. Our results indicate that hummingbirds exert similar selection on *P. brandegeei*.

Although our experimental arrays detected instances of strong floral selection by pollinators, natural field conditions may moderate these patterns. Visitation by pollen collecting insects and abiotic factors could change or obscure the selection we detected by hummingbirds in isolation. Also, the presence of other pollinators (e.g., hawkmoths) may alter the selection exerted by

hummingbirds if selection by the two pollinators is not additive (Sahli and Conner 2011). Furthermore, selection exerted on floral traits by *A. colubris* in our arrays may differ from selection by the observed pollinator *S. platycercus* in natural populations. Bill lengths for the two species are very similar but slightly longer in *S. platycercus*, so our estimates of selection for longer tubes and more exerted stigmas may be conservative. Although the ranges of *P. brandegeei* and *A. colubris* only slightly overlap, the overall patterns of selection between the two hummingbird species are likely similar, especially in comparison with selection by hawkmoths. Finally, resource limitation of seed set may be greater in natural populations, which may affect the magnitude of selection through female function. Plants in our arrays likely did not experience resource limitation, which could have enhanced pollen limitation and therefore selection through female function. Nevertheless, pollen limitation of seed set may be common in natural populations (Ashman and Morgan 2004). Despite these caveats, our results provide a baseline view of how hummingbirds contribute to selection on floral design in *P. brandegeei*.

Surrogate measures of pollinator visitation rates or estimates of pollen removal and deposition are required when direct fitness measures are unavailable. For example, large-scale plant community wide studies use visitation frequency to estimate plant reproduction (Alacórn 2010). However, these surrogate measures may not be reliable estimates of fitness. For example, the number of hummingbird visits a plant received over an array did not affect the number of pollen grains remaining in anthers. However, plants receiving more hummingbird visits sired more seeds than plants receiving fewer visits (Table 3). Furthermore, pollen deposition was not a good surrogate measure for the number of seeds set in the case of herkogamy (Table 2). Therefore, our study highlights the importance of directly measuring both female and male fitness.

SELECTION BY HAWKMOths AND HUMMINGBIRDS: IMPLICATIONS FOR FLORAL EVOLUTION AND TRAIT VARIATION

We measured selection on floral design by hummingbirds (this study) and hawkmoths (Kulbaba and Worley 2012) under controlled conditions on experimental arrays of *P. brandegeei*. The selection gradients we observed (Tables 2–4) reflected both the mechanics of pollen transport and the range of phenotypes in the arrays. First, hawkmoths transported pollen along a slender proboscis, and hummingbirds on their feathered forehead and chin regions (Kulbaba and Worley 2008; M. Kulbaba, pers. obs.). Second, the extremes in the distribution of herkogamy in the arrays were at higher frequencies than in natural populations, whereas the remaining floral traits reflected natural variation. Therefore, this experimental design enhanced our power to detect selection on herkogamy by each pollinator. We were most likely to detect selection on other floral traits with mean values that were distinct from either the hummingbird or hawkmoth optimum.

Hummingbirds responded to different features of *P. brandegeei* flowers than did hawkmoths in our previous study. Although hummingbirds in this study did not respond to variation in *P. brandegeei* floral dimensions, hawkmoths initially visited wider corollas but ultimately visited more plants displaying reverse herkogamy (Kulbaba and Worley 2012). Increased visitation frequency directly enhanced both female and male fitness under hawkmoth pollination, but only male fitness under hummingbird pollination.

We detected contrasting selection by hawkmoths and hummingbirds on herkogamy in *P. brandegeei* through female function. Hummingbirds selected for stigmas exerted beyond anthers (approach herkogamy) and hawkmoths selected for stigmas recessed below anthers (reverse herkogamy). Furthermore, we previously documented temporal fluctuation in the relative abundances of these pollinators (Kulbaba and Worley 2008). Such fluctuations in population size seem to be characteristic of hawkmoths (Campbell et al. 1997; Brunet 2009). Our findings illustrate how different pollinators may exert contrasting selection on the same target of floral design, resulting in an “intermediate” phenotype for that trait (cf. Aigner 2001; Sahli and Conner 2011). Indeed, most individuals of *P. brandegeei* displayed intermediate measures of herkogamy in natural populations (Kulbaba and Worley 2008). As hummingbirds were more frequently observed visiting *P. brandegeei* flowers (Kulbaba and Worley 2008), instances of selection for approach herkogamy likely outnumber instances of selection for reverse herkogamy. Differences in pollinator frequency may explain our observed ratio of 80 : 20 approach to reverse herkogamous plants in natural populations (Kulbaba and Worley 2008).

Hummingbirds and hawkmoths selected on one common and one different corolla dimension. Although hummingbirds in this study selected for longer corolla tubes through male function and wider corolla tubes through female function, hawkmoths selected only for narrow corolla tubes through male function (Kulbaba and Worley 2012). All else being equal, these results indicate that corolla diameter should exhibit an intermediate “compromise” phenotype, and corolla length increase toward the optimal phenotype for hummingbird pollination. However, given the strong positive genetic correlation between corolla length and width ($r_a = 0.997$; $P < 0.0001$; Kulbaba and Worley 2008), these two traits are unlikely to respond independently to selection (cf. Ashman and Majetic 2006). Hummingbirds appear to be more frequent visitors in the populations we have observed and corolla dimensions (length and width) in *P. brandegeei* flowers are generally more reflective of other taxa pollinated by hummingbirds than of those pollinated by hawkmoths (Kulbaba and Worley 2008). However, our detection of significant selection by hummingbirds on tube diameter and length indicates that neither trait is optimal for hummingbird pollination, perhaps because of the combined effects of a strong genetic correlation and

contrasting selection by hawkmoths and hummingbirds on tube diameter.

Differing patterns of selection across gender functions within hermaphroditic plants have been hypothesized to be common (Morgan 1992; Morgan and Schoen 1997). However, limited empirical support has been found for this prediction (Ashman and Morgan 2004). In an extensive study measuring selection by four pollinator groups through female and male function on *Raphanus raphanistrum*, Sahli and Conner (2011) detected significant differences in the direction of selection between female and male selection on two floral traits (anther exertion and flower number), but not in the magnitude of gender specific selection. Although our results partially complement the findings of Sahli and Conner (2011), they differ in that we found selection through female function was stronger than through male function.

The combined results of our array experiments that measured selection by hummingbirds and hawkmoths on *P. brandegeei* floral design illustrate the potential of floral designs to display both intermediate phenotypes and traits that are apparently specialized to one pollinator. Herkogamy likely represents an intermediate condition that reflects contrasting selection by both pollinators. Conversely, the corolla dimensions of *P. brandegeei* are either more important to one pollinator, or the corolla trait distributions are sufficiently functional over a wide range for both hummingbird and hawkmoth pollination (cf. Aigner 2001). Our results add to the few studies examining selection by multiple pollinators. However, further work is required on a wider range of pollinators and plant taxa, to gain a more general view of pollinator–floral trait interactions in species pollinated by multiple functional groups.

ACKNOWLEDGMENTS

The authors thank R. Currie, B. Ford, M. Piercey-Normore, and R. Westwood for suggestions throughout this study. L. Harder provided valuable advice and discussions regarding experimental design and analysis. The authors also thank L. Delph, S. Kalisz, M. Johnston, and the extremely helpful comments from two anonymous reviewers. This work is funded by an NSERC Discovery grant to ACW, and a Dafoe Graduate Fellowship (University of Manitoba) to MWK. The authors state no conflicts of interest.

LITERATURE CITED

- Aigner, P. A. 2001. Optimality modelling and fitness trade-offs: when should plants become pollinator specialists? *Oikos* 95:177–184.
- . 2004. Floral specialization without trade-offs: optimal corolla flare in contrasting pollination environments. *Ecology* 85:2560–2569.
- Alarcón, R. 2010. Congruence between visitation and pollen-transport networks in a California plant-pollinator community. *Oikos* 119:35–44.
- Alexandersson, R., and S. D. Johnson. 2002. Pollinator-mediated selection on flower-tube length in a hawkmoth-pollinated *Gladiolus* (Iridaceae). *Proc. R. Soc. Lond. B* 269:631–636.
- Ashman, T.-L., and C. J. Majetic. 2006. Genetic constraints on floral evolution: a review and evaluation of patterns. *Heredity* 96:343–352.
- Ashman, T.-L., and M. T. Morgan. 2004. Explaining phenotypic selection on plant attractive characters: male function, gender balance or ecological context? *Proc. R. Soc. Lond. B* 271:553–559.
- Baker, H. G. 1972. Sugar concentration in nectars from hummingbird flowers. *Biotropica* 7:37–41.
- Barrett, S. C. H. 2002. Sexual interference of the floral kind. *Heredity* 88:154–159.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2:349–368.
- Beattie, A. J. 1971. A technique for the study of insect-borne pollen. *Pan-Pac Entomol.* 47:82.
- Brown, G. S., and C. L. Gass. 1993. Spatial association learning by hummingbirds. *Anim. Behav.* 46:487–497.
- Brunet, J. 2009. Pollinators of the Rocky Mountain columbine: temporal variation, functional groups and associations with floral traits. *Ann. Bot.* 103:1567–1578.
- Campbell, D. R. 1996. Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution* 50:1442–1453.
- . 2009. Using phenotypic manipulations to study multivariate selection of floral traits associations. *Ann. Bot.* 103:1557–166.
- Campbell, D. R., N. M. Waser, and M. V. Price. 1996. Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. *Ecology* 77:1463–1472.
- Campbell, D. R., N. M. Waser, E. J. Melendez-Ackerman. 1997. Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *Am. Nat.* 149:295–315.
- Castellanos, M. C., P. Wilson, and J. D. Thomson. 2004. “Anit-bee” and “pro-bird” changes during the evolution of hummingbird pollination in *Penstemon* flowers. *J. Evol. Biol.* 17:876–885.
- Conner, J. K. 2002. Genetic mechanisms of floral trait correlations in a natural population. *Nature* 420:407–410.
- Cronk, Q., and I. Ojeda. 2008. Bird-pollinated flowers in an evolutionary and molecular context. *J. Exp. Bot.* 59:715–727.
- Davidson, J. F. 1950. The genus *Polemonium* [Tournefort] L. *Univ. Calif. Publ. Bot.* 23:209–282.
- Delph, L. F., J. L. Gehring, F. M. Frey, A. M. Artz, and M. Levri. 2004. Genetic constraints on floral evolution in a sexually dimorphic plant revealed by artificial selection. *Evolution* 58:1936–1946.
- de Waal, C. 2010. Reproductive ecology of bird-pollinated *Babiana* (Iridaceae): floral variation, mating patterns and genetic diversity. MSc thesis, Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada.
- Fægri, K., and L. van der Pijl. 1972. The principles of pollination ecology. 3rd ed. Longman, Edinburgh.
- Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Ann. Rev. Ecol. Evol. Syst.* 35:375–403.
- Forrest, J. R. K., J. E. Ogilvie, A. M. Gorischeck, and J. D. Thomson. 2011. Seasonal change in a pollinator community and the maintenance of style length variation in *Mertensia fusiformis* (Boraginaceae). *Ann. Bot.* 108:1–11.
- Gass, C. L., and G. D. Sutherland. 1985. Specialization by territorial hummingbirds on experimentally enriched patches of flowers: energetic profitability and learning. *Can. J. Zool.* 63:2125–2133.
- Grant, V. 1983. The systematic and geographical distribution of hawkmoth flowers in the temperate North American flora. *Bot. Gazette* 144:439–449.
- . 1985. Additional observations on temperate North American hawkmoth flowers. *Botanical Gazette* 146:517–520.
- Grant, K. A., and V. Grant. 1968. Hummingbirds and their flowers. Columbia Univ. Press, New York.

- Grant, V., and E. J. Temels. 1992. Foraging ability of rufous hummingbirds on hummingbird flowers and hawkmoth flowers. *Proc. Natl. Acad. Sci. U.S.A.* 89:9400–9404.
- Harder, L. D., S. C. H. Barrett, and W. W. Cole. 2000. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proc. R. Soc. Lond. B* 267:315–320.
- Harder, L. D., C. Y. Jordan, W. E. Gross, and M. B. Routley. 2004. Beyond floricism: the pollination function of inflorescences. *Plant Species Biol.* 19:137–148.
- Hereford, J., T. F. Hansen, and D. Houle. 2004. Comparing strengths of directional selection: how strong is strong? *Evolution* 58:2133–2143.
- Hodgins, K. A., and S. C. H. Barrett. 2008. Asymmetrical mating patterns and the evolution not biased morph ratios in a tristylous daffodil. *Genet. Res.* 90:3–15.
- Hurly, T. A. 2003. Twin-threshold model: risk-intermediate foraging by rufous hummingbirds, *Selasphorus rufus*. *Anim. Behav.* 66:751–761.
- Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16:1199–1006.
- Kearns, C. A., and D. W. Inouye. 1993. *Techniques for pollination biologists*. The Univ. Press of Colorado, Niwot.
- Kulbaba, M. W. and A. C. Worley. 2008. Floral design in *Polemonium brandegei* (Polemoniaceae): genetic and phenotypic variation under hawkmoth and hummingbird pollination. *Intl. J. Plant Sci.* 169:509–522.
- . 2011. Polymorphic microsatellite loci in *Polemonium brandegeei* and *P. viscosum* (section *Melliosoma* Polemoniaceae). *Am. J. Bot.* 98:e4–e6.
- . 2012. Selection on floral design in *Polemonium brandegeei* (Polemoniaceae): female and male fitness under hawkmoth pollination. *Evolution* 66:1344–1359.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Lertzman, K. P., and C. L. Gass. 1983. Alternative models of pollen transfer. Pp. 474–489 in C. E. Jones and R. J. Little, eds. *Scientific and Academic Editions*, New York.
- Meagher, T. R. 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of the most-likely male parents. *Am. Nat.* 128:199–215.
- Mitchell, R. J. 1993. Adaptive significance of *Ipomopsis aggregata* nectar production: observation and experiment in the field. *Evolution* 47:25–35.
- Mitchell, R. J., and N. M. Waser. 1992. Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. *Ecology* 73:633–638.
- Morgan, M. T. 1992. The evolution of traits influencing male and female fertility in outcrossing plants. *Am. Nat.* 139:1022–1051.
- Morgan, M. T., and D. J. Schoen. 1997. The role of theory in an emerging new plant reproductive biology. *Trends Ecol. Evol.* 12:231–234.
- Muchhala, N. 2007. Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *Am. Nat.* 169:449–504.
- Nilsson, L. A. 1988. The evolution of flowers with deep corolla tubes. *Nature* 384:147–149.
- Nishizawa, T., Y. Watano, E. Kinoshita, T. Kawqahara, and K. Ueda. 2005. Pollen movement in a natural population of *Arisaema serratum* (Araceae), a plant with a pitfall-trap flower pollination system. *Am. J. Bot.* 92:1114–1123.
- Ollerton, J. 2006. “Biological Barter”: patterns of specialization compared across different mutualisms. Pp. 411–435 in N. M. Waser, and J. Ollerton, eds. *Plant-pollinator interactions: from specialization to generalization*. University of Chicago Press, Chicago, IL.
- Pyle, P. 2001. *Identification guide to North American birds, Part I Columbidae to Ploceidae*. Slate Creek Press, Bolinas, CA.
- Reynolds, R. J., M. R. Dudash, and C. B. Fenster. 2009. Multiyear study of multivariate linear and nonlinear phenotypic selection on floral traits of hummingbird-pollinated *Silene virginica*. *Evolution* 64:358–369.
- Roff, D. A. 1997. *Evolutionary quantitative genetics*. Chapman and Hall, New York.
- Sahli, H. F., and J. K. Conner. 2011. Testing for conflicting and nonadditive selection: floral adaptation to multiple pollinators through male and female fitness. *Evolution* 65:1457–1473.
- SAS Institute. 2004. *SAS/STAT*, release 9.1.2. SAS Institute, Cary, NC.
- Schemske, D. W., and C. C. Horvitz. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* 225:519–521.
- Schluter, D., and D. Nychka. 1994. Exploring fitness landscapes. *Am. Nat.* 143:597–616.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd ed. W.H. Freeman and Co., New York.
- Stanton, M. L., A. A. Snow, and S. N. Handel. 1986. Floral evolution: attractiveness to pollinators increases male fitness. *Science* 232:1625–1627.
- Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms I: pollination mechanisms. *Ann. Rev. Ecol. Syst.* 1:307–326.
- Temeles, E. J. 1996. A new dimension to hummingbird-flower relationships. *Oecologia* 105:517–523.
- Temeles, E. J., C. R. Koulouris, S. E. Sander, and W. J. Kress. 2009. Effect of flower shape and size on foraging performance and trade-offs in a tropical hummingbird. *Ecology* 90:1147–1161.
- Waser, N. M., L. Chittka, M. V. Price, N. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77:1043–1060.
- Webb, C. J., and D. G. Lloyd. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. *Herkogamy*. *New Zealand J. Bot.* 24:163–178.
- Wilson, P., and E. Jordan. 2009. Hybrid intermediacy between pollination syndromes in *Penstemon*, and the role of nectar in affecting hummingbird visitation. *Botany* 87:272–282.

Associate Editor: M. Johnston

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Summary of replicate array populations, and number of identified hummingbird visitors by gender. Values within a column denoted by different letters are significantly different at $P < 0.05$ as determined by t -test. Measures are counts, except for Average Handling Time of Flowers that is reported in seconds.