PLANT ANIMAL INTERACTIONS

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Hummingbird foraging position is altered by the touch-sensitive stigma of bush monkeyflower

Abstract The two stigma lobes of bush monkeyflower (*Mimulus aurantiacus*) close together rapidly in response to touch by a hummingbird pollinator and usually remain closed for the life of the flower, preventing further pollen receipt. Previous work showed that hummingbirds visiting bush monkeyflowers with closed stigmas export more than twice as much pollen to recipient flowers as birds visiting flowers with open stigmas. To investigate how stigma closure increases pollen export, we used videotape to examine the interaction between bird and flower. Hummingbirds altered their method of foraging in response to whether the stigma was open or closed. When stigmas were closed, birds most frequently entered flowers from above, relative to the long axis of the corolla tube, bringing the bill close to the ceiling of the corolla where anthers and stigma are positioned. However, birds were much more likely to enter corollas from below when stigmas were open, keeping the bill farther from the sex organs, and resulting in failure to contact open stigmas 33% of the time. Distance of the bill from the corolla-tube ceiling was associated with frequency of contact with anthers and, when contact was made, explained pollen export in a non-linear fashion. Morphometric analyses showed that smaller corolla openings promoted stigma contact, but corolla openings that were wide relative to their height produced increased contact with anthers. Changes in hummingbird foraging position and reduced pollen export when visiting flowers with open stigmas helps to explain the observation of permanent stigma closure, even when less than full seed set has been achieved.

Keywords Herkogamy · Hummingbird · *Mimulus* · Pollen-pistil interference · Pollinator behavior

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Introduction

The animal pollination of a flower is a prime example of a mutualistic relationship between two organisms (Feinsinger 1983). The flower requires a vector to import and export pollen, and the pollinator plays this role only in return for an expected reward. The prevalence of nectar robbery throughout the angiosperms (Inouye 1983), in which nectar foragers "cheat" and bypass the sex parts to obtain a reward more efficiently, demonstrates the conflict of interest inherent in this mutualism. Despite this, there are advantages to employing animals as vectors because they nonrandomly direct pollen to other plants and, if pollinators selectively visit only a restricted number of species, they can increase pollen exchange between conspecifics.

Hermaphroditic flowers may improve the possibility that an animal foraging for rewards acts as a pollinator by structural or mechanistic contrivances that facilitate forager contact with anthers and/or stigmas during a visit (Murcia 1990; Connor et al. 1995; Hurlbert 1996; Smith et al. 1996; Cresswell 1998). This may be achieved by directing or by constraining foragers to probe the flower in an orientation such that they contact floral sex parts. Evolutionarily "advanced" features like bilateral symmetry, fused petals, and a positioning of the flower parallel to the ground have the additional benefit of promoting the deposition of pollen on a consistent part of the pollinator's body during each visit (Proctor et al. 1996, p. 154). Precise pollen placement on different body parts of a shared pollinator fauna can allow sympatric species to employ the same vector (Armbruster et al. 1994), while reducing the risks of heterospecific pollen deposition (Waser and Fugate 1986; Galen and Gregory 1989; Randall and Hilu 1990).

Precise contact of floral sex parts with flower visitors incurs a potential cost, however, because male and female floral functions are likely to interfere with each other. The potential for male-female (pollen-stigma) interference comes from the requirement for animal-pollinated flowers to place pollen on the pollinator's body in a position that

will contact the stigmas of subsequent flowers in order to effect transfer. Yet male and female organs cannot occupy the same space within a flower. Indeed, the closer the organs are to one another, the more likely one is to block the other's access to the pollinator's body.

Herkogamy, the spatial separation of anthers and stigmas within flowers, is very common, and is generally interpreted as an adaptation to reduce intrafloral self-pollination (Darwin 1876, p. 383). Before Darwin, however, Sprengel (1793, p. 18) recognized the potential for interference between floral sex functions and proposed that certain traits may be effective in curbing interference. More recently, Webb and Lloyd (1986) suggested that, in addition to herkogamy, other common floral characters such as temporal separation of stigma receptivity and pollen release (i.e. dichogamy, Lloyd and Webb 1986) and floral unisexuality, may evolve as mechanisms that reduce interference between male and female functions within the flower, rather than as selfing-avoidance mechanisms. In addition to this, secondary pollen presentation, whereby the pollen is available for export from a location within the flower distinct from the anthers, can be effective at curbing male-female interference (Yeo 1993).

In coastal southern California, USA, bush monkeyflower, *Mimulus aurantiacus* (Scrophulariaceae), has long, red, tubular flowers which are primarily hummingbird-pollinated. The single style and four filaments of bush monkeyflower are appressed to the ceiling of the corolla. The stigma projects slightly forward of the anthers and is composed of two sensitive lobes which close together rapidly (in about 2 s) in response to touch. The stigmatic lobes are open and receptive to pollen at anthesis, while the anthers begin to open about 1 day later. Reopening of the stigma following touch is a function of pollen receipt and ovule fertilization. If no pollen is received, the stigma always reopens within 5 h. Pollinated stigmas usually remain closed for the life of the flower, but are capable of reopening, receiving more pollen, and setting additional seed, if few ovules are fertilized. However, stigmas consistently remain permanently closed if enough ovules have been fertilized to achieve >one-third of maximum seed set (Fetscher and Kohn 1999).

Webb and Lloyd (1986) proposed that "movement herkogamy", in which organs of one sex move out of the way of the others following pollen receipt, is an adaptation to reduce intersexual conflict. Movement herkogamy allows each of the sex organs to contact the same part of the pollinator in turn, reducing interference between them. Webb and Lloyd (1986) included stigma closure as an example of movement herkogamy because additional spatial separation between floral sex organs is gained by moving the stigmatic lobes out of the way of the anthers in response to pollen deposition. They hypothesized that stigma closure allowed for increased pollen export from the flower. This was found to be true in bush monkeyflower, where hummingbirds visiting flowers with closed stigmas export 2.4 times more pollen to stigmas of subsequent flowers than do flowers with open stigmas (Fetscher 2001).

Manipulative experiments that involve varying a trait of interest represent an ideal way to study the trait's adaptive significance (Waser 1983; Campbell et al. 1994). The closeable stigma of bush monkeyflower is well suited to the investigation of the mechanisms of intersexual interference, because the two stigmatic "states", open and closed, both occur naturally and are inducible and reversible within flowers. In this study, we use manipulations of the stigma of bush monkeyflower, measurements of floral architecture, and observations of hummingbird-flower interactions, to examine the mechanism by which closure of the stigma improves pollen export. We ask the following questions:

1. Does the state of the stigma influence whether and how hummingbirds enter flowers?
2. How do changes in hummingbird behavior affect the amount of pollen exported?
3. What aspects of floral morphology improve hummingbird contact with floral sex organs, and therefore components of male and female fitness?

Materials and methods

All data were collected at Dawson-Los Monos Canyon Reserve in Vista, California, during July and August 1996–1998 using bush monkeyflower (*Mimulus aurantiacus* (Scrophulariaceae)), a self-compatible, chaparral shrub. A large population of bush monkeyflower occurs at this site. The hummingbird species visiting bush monkeyflower at the reserve were *Calypte anna* (Anna's), *Selasphorus sasin* (Allen's), *Calypte costae* (Costa's), *Archilochus alexandri* (Black-chinned), *Selasphorus rufus* (rufous), and rarely, *Stellula calliope*, (Calliope).

Hummingbirds were provided artificial inflorescences at which to forage. These consisted of a branched, upright piece of wood to which were attached florist's vials, each containing one bush monkeyflower. In size and shape, the artificial inflorescence resembled as closely as possible a real bush monkeyflower shrub. To stock the inflorescences, we harvested flower buds which were to open the following day from a large number of genets from the surrounding population of bush monkeyflower and kept them in water-filled trays lined with an Oasis foam block. Ten microliters of a 0.8 M sucrose solution was deposited inside the base of each flower with a micropipet to reflect the amount and concentration of nectar observed in unvisited flowers in the field (A.E. Fetscher, unpublished data), because flowers picked at the bud stage subsequently produce very little nectar.

To attract pollinators to the experimental setup, a hummingbird feeder was posted next to the artificial inflorescence starting 2 days before the beginning of each round of the study. We removed the feeder once we began running experimental foraging bouts at the artificial inflorescence. A new set of flowers was affixed to the artificial inflorescence for each hummingbird foraging bout. When a hummingbird arrived at the inflorescence and began foraging, we noted the order in which the flowers were visited and videotaped visits with a Sony Hi-8 Video Camcorder (model no.CCD-TR930) at 15× magnification.

To test for an effect of the state of the stigma (open vs closed) on hummingbird preference for flowers and components of handling time during foraging, we set up inflorescences with eight flowers. On half of the flowers stigmas were closed by touch before
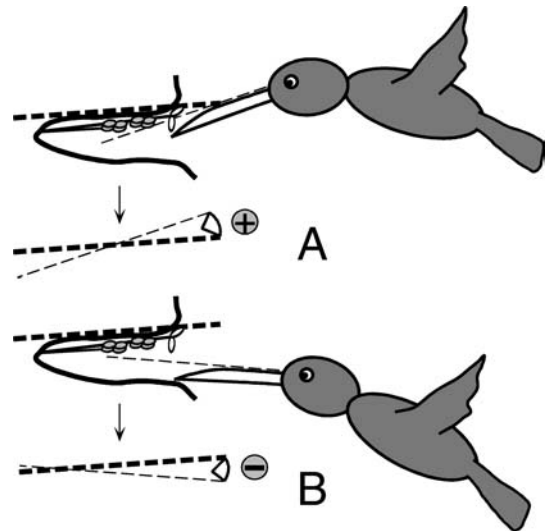
hummingbird visitation, while stigmas on the remaining flowers were left open. During each foraging bout, the inflorescence was videotaped so that all flowers were in view. From bout to bout, the positions of the open- and closed-stigma flowers on the inflorescence were alternated to remove any effects of position on pollinator behavior. The order of visitation of hummingbirds to flowers was noted, as were multiple visits to individual flowers. In addition, the duration the bird hovered in front of each flower before probing and the duration of each probe were measured.

To determine how aspects of floral morphology and the state of the stigma (open vs closed) affect hummingbird probing of a flower, contact with reproductive organs, and pollen export, inflorescences were constructed with one pollen donor and five recipient flowers. Only flowers whose anthers had fully dehisced (2 days after anthesis) were used as pollen donors. Donor flowers received one of two stigmatic treatments. The stigma was either left open or touched by hand to cause closure immediately prior to use. Recipient flowers were emasculated and their stigmas were left open and free of pollen. The order of visitation to flowers during each foraging bout was noted and visits to the donor flower were videotaped at close range.
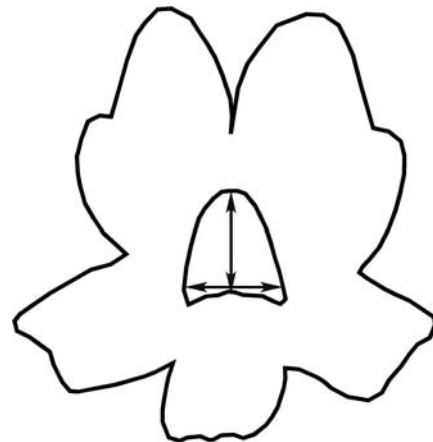
After each foraging bout, we examined all flowers that had been visited to assess contact between the hummingbird and floral sex parts. State of stigmas (open vs closed) was scored for all visited recipient flowers, as well as donor flowers from the open-stigma treatment. Stigma contact was assumed to have occurred when stigma closure followed hummingbird probing of a flower. For donor flowers only, anthers were examined to determine whether they had dispensed pollen. Upon touch by a pollinator, bush monkeyflower anthers show a longitudinal split devoid of pollen. The stigmas of recipient flowers visited subsequent to contact with donor flower anthers were collected and preserved in 1.5-ml Eppendorf tubes containing wicks soaked in FAA (Kearns and Inouye 1993). In order to count the number of pollen grains deposited on recipient stigmas, a drop of 0.1% aniline blue in 0.15 M K_2HPO_4 was added to each stigma on a microscope slide, upon which the stigma was flattened with a cover slip. Stained stigmas were then viewed for pollen counts under a Zeiss Axioskop 20 using fluorescence light microscopy.

We measured aspects of hummingbird foraging behavior at the donor flower (angle of entry into flower, distance of bill from the ceiling of the corolla at deepest probe, depth of probe at its deepest, and duration of probe), by playing back each visit, frame-by-frame, using a Sony VHS recorder (model no. SLV-R1000), on which one frame represents $\frac{1}{30}$ s. Data on hummingbird orientation while probing flowers were collected from two frames for each recorded foraging bout: frame 1 (FR 1), the first frame in which the tip of the bill was inserted in the corolla tube, and frame 2 (FR 2) the point during a visit at which the bill was inserted at its deepest. Frames were converted into Canvas files, and lines to be used in measurements were superimposed on the images. Measurements of these lines were then taken using Image Pro Plus. From FR 1, a line was drawn along the top of the corolla tube from proximal to distal ends, and another line was drawn along the top of the bird's bill. The angle formed at the intersection of these lines was measured and designated "angle of entry" (Fig. 1). We assigned this measure a negative value if the line along the bill fell below the line along the top of the corolla, at its throat, and a positive value if the line along the bill was above the line along the top of the corolla. The distance of the bill from the ceiling at the throat of the corolla in FR 2 was also measured. Floral measurements taken in the field for each bout were used to scale hummingbird morphological and body-position measurements on video.

To assess how floral morphology affects contact with sex organs and the efficiency of pollen transfer, we used calipers to measure each visited flower immediately following each hummingbird visit to an artificial inflorescence. The following measurements were taken: corolla-tube length, style length, closed-stigma length and width (the distances of a stigmatic lobe from its base to its tip and from side to side, respectively), and corolla-opening height and width (Fig. 2). The following composite variables were calculated: stigma area = 2 × closed-stigma length ×

Fig. 1 Angle-of-entry measurements taken from videotaped visits of hummingbirds to pollen-donor flowers on artificial inflorescences. Measurements were taken from FR 1 of the videotape recording of visits to pollen-donor flowers. *Lines* were drawn from the top of the corolla and the top of the bird's bill at the point of entry. The angle between the lines was measured to determine angle of entry. *A* and *B* show "positive" and "negative" angles of entry
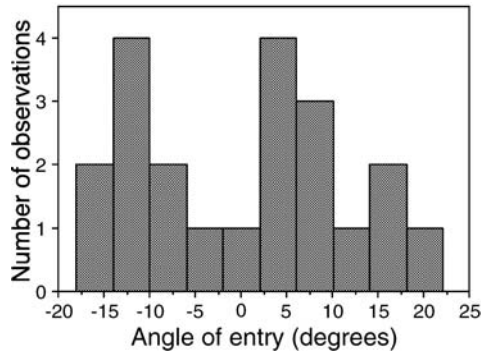

Fig. 2 Head-on view of a bush monkeyflower corolla without sex parts. The *vertical* and *horizontal arrows* show the measurements of corolla-opening height and width

stigma width, corolla-opening area = corolla-opening height × corolla-opening width, proportion eclipsed (the proportion of the corolla-opening area that was eclipsed by the open stigma) = open-stigma area / corolla-opening area, and corolla-opening shape = corolla-opening height – corolla-opening width. In addition to recording floral measurements, the state of the stigma (open or closed) of the donor flower was noted.

Table 1 Components of hummingbird foraging behavior at flowers with open and closed stigmas
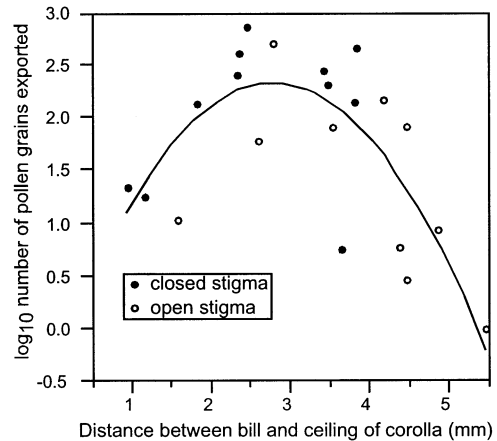
Behavioral component	State of the stigma		Effect
	Open	Closed	
Hover time (s): $\bar{x}$ (SE), n	0.20 (0.03), 133	0.18 (0.02), 130	F=0.1481; P>0.7
Duration of probe (s): $\bar{x}$ (SE), n	1.20 (0.05), 172	1.21 (0.05), 160	F=0.0402; P>0.8
Depth of probe (mm): $\bar{x}$ (SE), n	7.28 (0.28), 22	7.27 (0.23), 16	F=0.0100; P>0.9
Visit-order rank: $\bar{x}$ (SE), n	4.23 (0.19), 137	4.49 (0.20), 131	Z=0.9651; P>0.3
Flowers probed multiple times, n	20, 199	16, 194	χ^2=1.098; P>0.29
First flower visited, n=35	20	15	χ^2=1.429; P>0.23

Fig. 3 Frequency distribution of the angle at which hummingbirds entered pollen-donor flowers, from FR 1 of videotaped visits. n=42

Fig. 4 The relationship between pollen export and the distance between the hummingbird's bill at maximum insertion and the ceiling of the corolla. *Solid circles* correspond to pollen-donor flowers with closed stigmas, and *open circles* correspond to donors with open stigmas

Results

Question 1: Does the state of the stigma influence whether and how hummingbirds enter flowers?

Hummingbirds showed no detectable preference for flowers based on the state of their stigma (open vs closed) nor were any measured components of hummingbird behavior, including handling time, affected by donor-stigma treatment (Table 1). The state of the stigma did, however, affect the way in which flowers were visited. Overall, angles of entry were bimodally distributed (Fig. 3), and strongly affected by whether the stigma was open or closed. Since these data were bimodal and non-normalizable within treatments, we treated them as categorical (i.e., "positive-" and "negative angle of entry"). Hummingbird bills formed a negative angle relative to the ceiling of the corolla tube in a significantly higher proportion of visits to pollen-donor flowers whose stigmas were open (68%; n=22) than closed (35%; n=20; χ^2=4.624; P<0.05). The distance of the hummingbirds' bills from the ceiling of the corolla at deepest insertion was much greater if the angle of entry was negative than positive (negative $\bar{x}$ =3.9 mm; positive $\bar{x}$ =2.5 mm; F=8.1, P=0.01). This suggests that the angle at which a hummingbird orients itself as it enters a flower is related to the distance between the hummingbird's bill and the floral reproductive organs at full insertion. Overall, anthers were contacted by hummingbirds in 84.8% (n=46) of foraging bouts when the donor-flower stigma was closed and in 71.5% (n=53) of bouts when the donor's stigma was open, a difference that was not statistically significant (χ^2=2.5; P=0.11).

Question 2: How do changes in hummingbird behavior affect the amount of pollen exported?

In videotaped bouts during which contact with anthers was made, flowers with closed stigmas exported 2.8 times more pollen to recipient stigmas than did flowers with open stigmas (t-test on log-transformed data; t=2.192; df=19; P<0.05). These bouts represent a subset of the data analyzed in Fetscher (2001), but here we use only bouts from which both data on pollen export and clearly visible video footage of the interaction between hummingbird and flower were available. For videotaped bouts, distance of the bill from the ceiling of the corolla had a marginally significant effect on the frequency of contact with anthers, with smaller distances causing more frequent contact (n=43, χ^2=3.23; P<0.05, one-tailed). In visits in which contact with the anthers is made, neither entry angle nor distance of the bill from the top of the corolla at maximum insertion was a significant predictor of pollen export in linear models whether or not these models also contained the effect of stigma treatment. However, a strong and highly significant non-linear relationship, independent of treatment, was found between pollen export and the distance of the hummingbird's bill from the ceiling of the corolla at full insertion (Fig. 4, Table 2).

Table 2 Analysis of the relationship between the distance of the bill from the ceiling of the corolla at maximum insertion and pollen export (log-transformed). Neither stigma treatment (open vs closed) nor distance × stigma treatment were significant, and these effects were removed from the final model

Term	Estimate	Sum of squares	F-ratio	P	R^2
Intercept	−0.394	–	–	0.601	0.571
Distance	−1.971	5.026	15.104	0.011	
Distance^2	−0.354	6.474	19.456	0.003	

Table 3 Pearson correlation coefficients between floral traits in bush monkeyflower (n=199). Significance values are for direct pairwise correlations. After Bonferroni correction (15 tests), only correlations with $P<0.001$ are significant
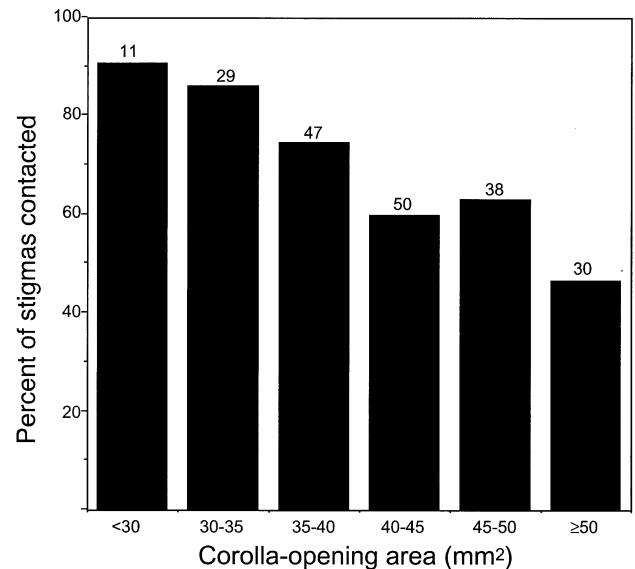
Character	Character				
	Style length	Stigma length	Stigma width	Corolla-opening height	Corolla-opening width
Corolla length	0.6492***	0.0016	−0.0020	0.1611*	0.1617*
Style length	–	0.0174	−0.0514	0.1422*	0.1232
Stigma length		–	0.2554***	0.0380	0.0518
Stigma width			–	0.1406	0.0793
Corolla-opening height				–	0.3580***

*$P<0.05$, ***$P<0.001$

Question 3: What aspects of floral morphology improve hummingbird contact with floral sex organs, and therefore components of male and female fitness?

Several of the floral traits measured were significantly positively correlated: corolla length with style length, corolla-opening height, and corolla-opening width, style length with corolla-opening height, stigma length with stigma width, and corolla-opening height with corolla-opening width (Table 3). While stigma height and width were correlated, each was independent of all other floral measures. After Bonferroni correction (Rice 1989) for the 15 pairwise tests (Table 3), only the correlations between corolla length and style length, stigma length and height, and corolla height and width remained significant.

Hummingbirds foraging at artificial inflorescences failed to contact open stigmas in 33% (n=204) of visits to recipient flowers. To explore which aspects of floral architecture influence contact with open stigmas, simple logistic regression was conducted on all floral morphometric data using contact with open stigmas as the response variable. For the six direct floral measures (corolla length, style length, stigma length and width, corolla opening height and width) the rate of stigma contact increased with narrower corolla openings in both vertical (n=204, χ^2=12.15; $P<0.0005$) and horizontal (χ^2=8.27; $P<0.004$) dimensions while none of the other measures were significant. For compound variables (stigma exsertion, stigma area, corolla opening area, corolla opening shape, and proportion of corolla opening blocked by the stigma), stigma contact increased with smaller corolla opening area (χ^2=14.58; $P<0.0001$; Fig. 5) and with a greater proportion of the corolla opening blocked by the stigma (χ^2=6.78; $P<0.01$). All floral morphological variables with significant univariate effects on stigma contact were loaded into the multiple logistic regression and least-significant factors were

Fig. 5 The frequency with which stigmas of pollen-recipient flowers were contacted by hummingbirds, causing closure, as a function of corolla-opening area (mm^2). *Numbers* above each *bar* are sample sizes. Each doubly-bounded interval of corolla opening area begins on the lower bound and extends up to, but does not include, the upper bound

iteratively removed until only significant factors remained. The penultimate model contained both corolla opening height and width, both of which were negatively related to stigma contact. However, the effect of corolla opening width was marginally non-significant ($P<0.07$) leaving only corolla opening height in the final model. Since corolla opening height, width, and area are all highly correlated and significant (negative) predictors of stigma contact, these results indicate that corolla opening size negatively affects stigma contact.

Corolla-opening shape (χ^2=4.63; P<0.05) and proportion eclipsed (χ^2=3.85; P<0.05) were the only significant univariate predictors of anther contact (n=99). When these factors were loaded into a multiple regression model, only corolla-opening shape remained in the final model. The wider the corolla opening relative to its height, the more likely anthers were to be contacted by hummingbirds. Although the effect of stigma treatment (open vs closed) on anther contact was not statistically significant (P=0.11, see above), it seemed logical to split the data by treatment because birds visited flowers with closed versus open stigmas differently, raising the possibility that different features might affect anther contact. For flowers with closed stigmas, only corolla opening shape had a significant effect on anther contact (n=46, χ^2=6.63; P<0.01). This effect was undetectable in flowers with open stigmas (n=53, χ^2=0.8, NS), nor were any other floral measures significant predictors of anther contact when stigmas were open. While the interaction between stigma treatment and corolla opening shape on anther contact is not significant (χ^2=2.7; P=0.1), the data suggest that corolla openings that are wide relative to their height promote anther contact in flowers with closed stigmas, but this effect is absent or difficult to detect in flowers with open stigmas.

Discussion

Hummingbird orientation when probing bush monkeyflowers is strongly influenced by whether the stigma is open or closed prior to a visit. Foragers are twice as likely to enter flowers at a negative angle relative to the ceiling of the corolla (Fig. 1) when the stigma is open than when it is closed. The angle of entry assumed by a hummingbird when entering a flower predicts the distance between the hummingbird's bill at maximum insertion and the ceiling of the corolla where sex organs are positioned. Hummingbirds often fail to contact open stigmas and the distance of the bill from the ceiling of the corolla affects the frequency of contact with anthers. When anthers are contacted, the amount of pollen exported and deposited on subsequent stigmas is more than twice as large if the stigma is closed than if it is open.

Why do hummingbirds alter their foraging position depending on the state of the stigma? We posit that pollinators actively avoid contact with stigmas. It is difficult to understand why birds would alter their body position except as an attempt to avoid interference by the stigma in their foraging. The alternative, that bill entry from below is the preferred method of handling flowers, but that this method needs to be altered to entry from above when stigmas are closed, makes little sense.

A number of factors might cause hummingbirds to avoid stigmas. Bush monkeyflowers can have a stigmatic surface area of as much as 13 mm^2, and the stigma is often exserted slightly beyond the opening of the corolla. Perhaps the stigma is large enough to interfere with the bird's vision when foraging or compromise its ability to

orient its body adequately for nectar acquisition by pressing against the feeding bird. Videotapes of birds visiting flowers with open stigmas have confirmed that, when a forager contacts an open stigma, the bird's forehead is generally pressed against it the whole time the bird is imbibing nectar (Fetscher 2001).

We explored the possibility that birds choose which flowers to probe based on the state of the stigma. Hummingbirds might favor flowers with closed stigmas over open if the former were easier to handle. Alternatively, since a closed stigma in nature means that the flower has already been visited, and might not contain nectar, birds might prefer flowers with open stigmas. Pollinators of other species have been shown to make foraging choices based on visual cues like flower color (Waser and Price 1981) and size (Cresswell and Galen 1991), but we found no evidence for any preference (Table 1). Either differences in flowers based on the state of the stigma are not large enough to affect preference, or the cost of probing a flower that has a closed stigma and may be depleted of nectar is balanced by some unknown locomotory cost to visiting flowers with open stigmas. We anticipated that hummingbird alteration of foraging behavior would prolong handling time when birds foraged at flowers with open stigmas. However, we were unable to detect any effect of the state of the stigma on any aspect of handling time, either before entering a flower or while feeding (Table 1). Perhaps, by changing probing behavior depending on the state of the stigma, birds are achieving equal forging efficiency on flowers of alternate stigma states.

Despite large differences in bird orientation and pollen export based on the state of the stigma, neither angle of entry nor distance of the bill from the ceiling of the corolla tube had a significant linear effect on the amount of pollen exported following contact with the anthers. Instead, a non-linear relationship between distance of bill from the ceiling of the corolla and pollen export was found. As expected, low levels of pollen export occurred when this distance was great. However, intermediate distances produced the highest pollen export to subsequent flowers, while only moderate pollen export occurred when the distance was small (Fig. 4). One possible explanation for less than maximal pollen export when birds forage with their bills near the ceiling of the corolla tube is that they alter their position laterally such that pollen pick-up is reduced. Such lateral variation in body position could not be detected by our filming (we filmed a side view of donor flowers and birds). However, because flowers with greater corolla tube width relative to height showed increased likelihood of anther contact, lateral movements are an unlikely cause of decreased pollen export. Another possibility is that pollen may be scraped off the bill by the style or the top of the corolla as birds retreat from flowers if their bill is close to the top of the corolla tube.

Stigmas of flowers with smaller corolla-opening areas were more likely to be contacted by hummingbirds (Fig. 5). Therefore, selection to increase the rate of

contact with the stigma should favor narrow corollas. Given that hummingbirds fail to contact open stigmas in a third of visits, selection for narrower corollas should be strong as flowers are often giving away nectar without pollen receipt. One explanation for the maintenance of current corolla-opening size may be opposing selection favoring male function (Campbell 1989). We found that flowers with a corolla-opening shape that tended toward increased width relative to height experienced a higher frequency of pollinator contact with anthers. Wider corollas have been found to improve male function in a number of species (Galen and Stanton 1989; Campbell et al. 1991, 1996; Smith et al. 1996), though the mechanism for this is poorly understood. If the observed positive phenotypic correlation between corolla-opening height and width (Table 3) reflects an underlying genetic correlation, the existing corolla-opening dimensions may be the product of stabilizing selection resulting from opposing male and female selective pressures.

The closed-stigma treatment used in this study reflects the situation in nature in which the flower has already received pollen and enough ovules have been fertilized for the stigma to remain permanently closed. Though the stigma is capable of reopening after pollination, it tends not to do so, precluding further pollen receipt, even when only enough ovules are fertilized for one-third of the maximum seed set (Fetscher and Kohn 1999). This behavior of the stigma is understandable in the context of the positive effect of stigma closure on male fitness. Even if far less than maximal female fitness has been realized, it may be advantageous for the flower to switch to a functionally male state and forgo potential further fitness gains through female function. Because visitation rates to flowers of this species are low, and because hummingbirds often fail to contact stigmas when visiting, many pollinated flowers would not receive a second load of pollen even in the absence of stigma closure. Fetscher and Kohn (1999) showed that naturally pollinated flowers whose stigmas were prevented from closure through experimental manipulation, received only slightly more pollen over their lifetime than did unmanipulated control flowers. This suggests that, even in the absence of stigma closure, most flowers would receive only one pollen-depositing visit in their lifetime. That bush monkeyflower exhibits protogyny, the form of dichogamy in which stigma receptivity precedes pollen release, can also be explained in terms of the effect of stigma closure on pollen export. The anthers withhold their pollen during the first day of the flower's life when, because of interference of the open stigma with pollen export, their contents are not likely to realize full export potential, and do not begin to expose their pollen until the stigma has had the opportunity to receive pollen and close.

This study provides evidence that hummingbird pollinators change position in response to whether bush monkeyflower stigmas are open or closed. This alteration of foraging behavior probably explains reduced pollen export from flowers with open stigmas and appears to result from pollinator avoidance of the open stigma. This behavior reduces female fitness because hummingbirds often fail to contact stigmas and deposit pollen. In bush monkeyflower, stigma closure following pollination reduces the conflict between male and female floral functions, brokering an effective relationship between flower and pollinator.

References

Armbruster WS, Edwards ME, Debevec AM (1994) Floral character displacement generates assemblage structure of western Australian triggerplants (*Stylidium*). Ecology 75:315–329

Campbell DR (1989) Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. Evolution 43:318–334

Campbell DR, Waser NM, Price MV, Lynch EA, Mitchell RJ (1991) Components of phenotypic selection: pollen export and floral corolla width in *Ipomopsis aggregata*. Evolution 45:1458–1467

Campbell DR, Waser NM, Price MV (1994) Indirect selection of stigma position in *Ipomopsis aggregata* via a genetically correlated trait. Evolution 48:55–68

Campbell DR, Waser NM, Price MV (1996) Mechanisms of hummingbird-mediated selection for floral width in *Ipomopsis aggregata*. Ecology 77:1463–1472

Conner JK, Davis R, Rush S (1995) The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. Oecologia 104:234–245

Cresswell JE (1998) Stabilizing selection and the structural variability of flowers within species. Ann Bot 81:463–473

Cresswell JE, Galen S (1991) Frequency–dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. Am Nat 138:1342–1353

Darwin C (1876) The effects of cross and self fertilization in the vegetable kingdom. Murray, London

Fetscher AE (2001) Resolution of male-female conflict in an hermaphroditic flower. Proc R Soc Lond B 268:525–529

Fetscher AE, Kohn JR (1999) Stigma behavior in *Mimulus aurantiacus* (Scrophulariaceae). Am J Bot 86:1130–1135

Feinsinger P (1983) Coevolution and pollination. In: Futuyma DJ, Slatkin M (eds) Coevolution. Sinauer, Sunderland, Mass. pp 282–310

Galen C, Gregory T (1989) Interspecific pollen transfer as a mechanism of competition: Consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. Oecologia 81:120–123

Galen C, Stanton ML (1989) Bumble bee pollination and floral morphology: factors influencing pollen dispersal in the alpine sky pilot *Polemonium viscosum* (Polemoniaceae). Am J Bot 76:419–426

Hurlbert AH, Hoso SA, Temeles EJ, Ewald PW (1996) Mobility of *Impatiens capensis* flowers: effect on pollen deposition and hummingbird foraging. Oecologia 105:243–246

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot, Colo.

Inouye DE (1983) The ecology of nectar robbing. In: Bentley B, Elias T (eds) The biology of nectaries. Columbia University Press, New York pp 153–173

Lloyd DG, Webb CJ (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. NZ J Bot 24:135–162

Murcia C (1990) Effect of floral morphology and temperature on pollen receipt and removal in *Ipomoea trichocarpa*. Ecology 71:1098–1109

Proctor M, Yeo P, Lack A (1996) The natural history of pollination. Timber Press, Portland, Ore.

Randall JL, Hilu KW (1990) Interference through improper pollen transfer in mixed stands of *Impatiens capensis* and *Impatiens pallida* (Balsaminaceae). Am J Bot 77:939–944

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Smith CE, Stevens JT, Temeles EJ, Ewald PW, Hebert RJ, Bonkovsky RL (1996) Effect of floral orifice width and shape on hummingbird-flower interactions. Oecologia 106:482–492

Sprengel CK (1793) Das entdecktde Geheimniss der Natur im Bau und in der Befruchtung der Blumen I. Vieweg sen. Berlin.

Reprint (1972) by Cramer J, Swann HK, Lehre, and by Wheldon and Wesley, Codicote, New York

Waser NM (1983) The adaptive nature of floral traits: ideas and evidence. In: Real LA (ed) Pollination biology. Academic Press, New York, pp 241–285

Waser NM, Fugate ML (1986) Pollen precedence and stigma closure: A mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. Oecologia 70:573–57

Waser NM, Price MV (1981) Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. Evolution 35:376–390

Webb CJ, Lloyd DG (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. NZ J Bot 24:163–178

Yeo PF (1993) Secondary pollen presentation: form, function and evolution (Plant systematics and evolution, Suppl 6). Springer, Berlin Heidelberg New York