Division of labour within flowers: Heteranthery, a floral strategy to reconcile contrasting pollen fates

Mario Vallejo-Marín*

Jessamyn S. Manson

James D. Thomson

Spencer C. H. Barrett

Department of Ecology & Evolutionary Biology. University of Toronto, 25 Willcocks Street, Toronto, Ontario, M5S 3B2. Canada.

*Author for correspondence. Present address: School of Biological and Environmental Sciences. University of Stirling. Stirling, FK9 4LA. Scotland, U.K. Tel. (+44) 01786 467840. email: mario.vallejo@stir.ac.uk

Running title: Division of labor within flowers

Abstract

In many nectarless flowering plants, pollen serves as both the carrier of male gametes and as food for pollinators. This can generate an evolutionary conflict if the use of pollen as food by pollinators reduces the number of gametes available for cross-fertilization. Heteranthery, the production of two or more stamen types by individual flowers reduces this conflict by allowing different stamens to specialize in "pollinating" and "feeding" functions. We used experimental studies of *Solanum rostratum* (Solanaceae) and theoretical models to investigate this "division-of-labour" hypothesis. Flight cage experiments with pollinating bumble bees (*Bombus impatiens*) demonstrated that although feeding anthers are preferentially manipulated by bees, pollinating anthers export more pollen to other flowers. Evolutionary stability analysis of a model of pollination by pollen consumers indicated that heteranthery evolves when bees consume more pollen than should optimally be exchanged for visitation services, particularly when pollinators adjust their visitation according to the amount of pollen collected.

Keywords: bee pollination, *Bombus impatiens*, evolutionary stable strategy, heteranthery, nectarless flowers, *Solanum rostratum*, stamen functions

[Regarding plants] with two kinds of anthers... I am very low about them, and have wasted enormous labour over them, and cannot yet get a glimpse of the meaning of the parts.

C. Darwin to J. D. Hooker, October 14, 1862

I have had a letter from Fritz Müller suggesting a novel and very curious explanation of certain plants producing two sets of anthers of different colour. This has set me on fire to renew the laborious experiments which I made on this subject, now 20 years ago.

C. Darwin to W. Thiselton-Dyer, March 21, 1881

Introduction

In many species of flowering plants pollen, the vehicle for the transport of male gametes during cross-fertilization, is also consumed by pollinators in exchange for pollination services. The loss of pollen may be especially significant in nectarless flowers where pollen represents the only floral reward for animal pollinators, e.g. buzz-pollinated species (Buchmann, 1983). This situation can have important evolutionary consequences when the exchange of pollen as food to attract pollinators lowers the total number of gametes available for cross-fertilization and reduces fitness. Investigation of potential adaptive solutions for reconciling these contrasting pollen fates in nectarless flowers has received relatively little attention in the literature on floral function and evolution (but see Harder, 1990a; Harder & Wilson, 1997; Luo et al., 2008a).

The production of two or more types of stamens in the same flower (heteranthery) may help to reduce the fitness costs arising from pollen consumption by pollinators by allowing different sets of stamens to specialize in "pollinating" and "feeding" functions. Heteranthery has evolved in more than 20 families and is commonly associated with bee-pollinated, nectarless flowers (Vogel, 1978; Buchmann, 1983; Endress, 1994; Jesson & Barrett, 2003). The stamens of heterantherous species usually differ in shape, size, or colour, with two types being most common. Typically one set of stamens has brightly coloured anthers and is easily accessible to visitors that collect pollen. The other stamens usually have different, often cryptically-coloured, anthers that are larger, and are usually displaced from the main floral axis to a position corresponding to the location of the stigma (Jesson & Barrett, 2003). Heteranthery provides an opportunity to investigate how differentiation in anther form and function may reduce the fitness costs of using male gametes as food for pollinators.

Anther dimorphism intrigued Charles Darwin for more than 20 years and was the object of one of his last scientific enquiries (Darwin, 1899; Buchmann, 1983). Yet, as indicated in the quotations above, he failed to provide a functional explanation for heteranthery, unlike the plethora of other floral adaptations that he investigated (Darwin, 1877). Although he suspected that the two sorts of anthers differed functionally, he was unable to determine what the different functions were (quotation above). The German naturalist Fritz Müller provided the first explanation regarding the function of heteranthery (see quotation above, and Müller, 1883). Based on observations of multiple heterantherous species, he and his brother Hermann Müller suggested that heteranthery represents anther specialization into “feeding” and “pollinating” types, whereby the former reward pollinators and the latter are directly involved in cross-pollination (H. Müller, 1881, 1882; F. Müller, 1883). Darwin (quotation above) immediately grasped the significance and plausibility of the Müllers’ proposal, and this “division-of-labor hypothesis” (F. Darwin, 1899) remains the most prevalent explanation for the functional significance of heteranthery (Vogel, 1978; Dulberger, 1981; Buchmann, 1983; Lloyd, 1992a;

Graham & Barrett, 1995; Endress, 1997; Lester et al., 1999; Jesson & Barrett, 2003; Marazzi et al., 2007; Ushimaru et al., 2007), sometimes misattributed to Darwin (Luo et al., 2008a,b). According to Muller's hypothesis, 1) pollinators focus their pollen-collecting efforts on "feeding" anthers, rather than on "pollinating" anthers, so that 2) pollen from "pollinating" anthers is more successful at reaching stigmas of other plants than pollen from "feeding" anthers.

Despite its early origins, the division-of-labor hypothesis has received few empirical tests, and most have not fully addressed both of its components (Bowers, 1975; Wolfe et al., 1991; Wolfe & Estes, 1992; Gross & Kukuk, 2001; Tang & Huang, 2007; Ushimaru et al., 2007; but see Luo et al., 2008a). Only one of three studies that tracked dispersal of dye or metal particles applied to anthers of heterantherous species [*Solanum rostratum* (Solanaceae), *Chamaecrista fasciculata* (Caesalpiniaceae)] found greater dispersal from pollinating anthers than feeding anthers (Bowers 1975; Wolfe et al. 1991; Wolfe and Estes 1992). Whether these pollen surrogates applied to the exterior of anthers are good analogues of pollen grains in buzz-pollinated species, such as *Solanum* or *Chamaecrista,* is debatable. Tang and Huang (2007) reported that removal of feeding anthers from *Monochoria korsakowii* (Pontederiaceae) reduced visitation by pollinators and that flowers lacking pollinating anthers exported less pollen than flowers without feeding anthers. However, interpretation of their results is complicated, because Tang and Huang (2007) could not distinguish between self- and cross-pollen deposition on stigmas, and because anther removal introduced differences in the total number of pollen grains available for export. Other studies addressing the effect of removal of feeding anthers on pollinator visitation found either marginal effects [(*Commelina communis* (Commelinaceae), Ushimaru et al., 2007)], or did not compare the number of visits among treatments statistically [(*Melastoma affine* (Melastomataceae), Gross & Kukuk, 2001)]. To date the strongest support for the division of labor hypothesis comes from a study by Luo et al. (2008a) on *Melastoma malabatrichum* (=*M. affine*). They showed that pollen from pollinating anthers is more likely to land on stigmas of other flowers than pollen from feeding anthers and that removal of feeding anthers but not pollinating anthers reduced pollinator visitation. However, their estimate of pollen deposition on stigmas did not account for differences in pollen production and, potentially, pollen removal from the two types of anthers. Currently, the division of labor is the most plausible hypothesis for the function of heteranthery, but the hypothesis clearly requires further investigation.

Anther polymorphisms have also been investigated theoretically. Lloyd (2000) pointed out that heteranthery could be interpreted as the functional sterilization of part of the gametes (feeding anther pollen) to benefit the remaining gametes (pollinating anther pollen). He showed that a parentally-expressed gene causing the production of sterile pollen will increase in frequency if the benefits of producing reproductively disabled pollen exceed the costs ($b > c$), whereas a doubling of the relative benefits is necessary when the disability gene is expressed in offspring ($b > 2c$). Although Lloyd's model represents a valuable first step in understanding the evolution of heteranthery, models that explicitly incorporate the pollination process (e.g. Harder & Barrett, 1996) are necessary to understand the specific characteristics of both plants and pollinators that facilitate the evolution of heteranthery.

Here we combine experimental and evolutionary stable strategy (ESS) models (Lloyd, 1979; Maynard-Smith, 1982; Morgan, 2006) to address two main questions: (1) Is anther dimorphism accompanied by division of labour between anther types? (2) Is division of labour sufficient to favour the evolutionary maintenance of heteranthery, and if so, how is it affected by plant and pollinator characteristics? For our experimental test, we used captive bumble bees

(*Bombus impatiens* Cresson) visiting flowers of *Solanum rostratum* L. (Solanaceae). Specifically, we established experimentally whether feeding and pollinating anthers differ in pollinator attraction by disabling all anthers of the same type so that their pollen was inaccessible, and then recording bee behaviour. Because we did not alter flower morphology, we predicted no difference in the number of visits to flowers with blocked versus unmanipulated anthers. In contrast, because bees actively respond to differences in pollen availability (Buchmann & Cane, 1989), we predicted they would perceive flowers without functional feeding anthers as unrewarding, and therefore spend less time buzzing them compared to flowers without functional pollinating anthers or unmanipulated flowers. We also determined whether pollen from pollinating anthers is more likely to be transported by bees to stigmas of other flowers than pollen from feeding anthers. For our theoretical investigation of heteranthery, we modeled the pollination process of a species with dimorphic anthers visited by pollen-collecting insects to determine the conditions required to maintain heteranthery. Our analysis suggests that stamen dimorphism within flowers represents a floral strategy to minimize the fitness costs arising from the trade-off between using pollen as both a reward to attract pollinators and as gametes for cross-pollination.

Material and Methods

Study system

Solanum species produce pollen as the only reward for pollinators and are buzz pollinated, usually by bumble bees (Buchmann, 1983). The vast majority of the ~1500 *Solanum* species produce a single type of anther; however, within the spiny *Solanum* (subgenus *Leptostemonum*) heteranthery has evolved independently at least three times (Levin et al., 2006) . We studied

Solanum rostratum L. (Sect. *Androceras*), a widespread North American, annual, self-compatible species with weakly bilaterally symmetric, yellow enantiostylous flowers (Whalen, 1979). Individual plants produce two types of flowers with either a right- or left-deflected style and a single, large, brown pollinating anther positioned in the opposite direction (Todd, 1882; Harris & Kuchs, 1902; Bowers, 1975; Jesson & Barrett, 2002, 2005). The remaining four bright yellow feeding anthers are centrally located within the flower. No difference in the fertility of pollen produced by the two types of anthers has been detected in *S. rostratum* (Bowers 1975). Diverse insects visit flowers, including bees, wasps and flies, although *Bombus* spp. (including *B. impatiens*) are the primary pollinators (Bowers, 1975; Jesson & Barrett, 2005). *Bombus* spp. visiting *S. rostratum* typically grab the feeding anthers with their mandibles, vigorously vibrating their indirect flight muscles (“buzzing”). This results in all anthers, including pollinating anthers, releasing pollen through their apical pores (Bowers, 1975).

To investigate pollen traits of anther types, we grew *S. rostratum* (accessions #804750199, #904750111, #984750086; Nijmegen Botanical Gardens, Radboud University, Netherlands) in a 3:1 mix of soil:sand in 15.2-cm plastic pots and fertilized them with 13:13:13 slow-release granular fertilizer. We grew plants in a glasshouse with 16 h light at 25 ºC. We analyzed variation in pollen size (diameter) and pollen number using a linear mixed effects model fitted via restricted maximum likelihood (*nlme* package, R-Development Core Team, 2008). All statistical analysis used the program *R ver.* 2.6.2 (R-Development Core Team, 2008).

Pollinator attraction and behavior

The division-of-labour hypothesis states that feeding anthers principally attract pollinators. Unlike previous studies, which altered both visual cues and reward availability (e.g. Tang &

Huang, 2007; Ushimaru et al., 2007), we manipulated pollen availability in pollinating and feeding anthers without affecting visual cues or floral morphology. Specifically, we prevented pollen release by sealing the anther pores with a tiny amount of polyvinyl acetate glue, which simulates empty, unrewarding anthers (Buchmann & Cane, 1989). We then tested differential responses of bumble bees (*Bombus impatiens*) to feeding and pollinating anthers by: (1) recording the number and duration of visits to flowers in an experimental array, and (2) examining direct manipulation of each anther type by pollinators on individual flowers. We predicted that restricting access to pollen of feeding anthers would reduce pollinator attraction, whereas preventing access to pollen in pollinating anthers would not affect pollinator behaviour.

We used bees from two commercial colonies (Biobest Canada, Ltd; Leamington, Ontario) and a 3 x 3.3 x 2 m flight cage. For each trial, we collected nine flowers of similar size and allocated them to three treatments: control (C), feeding anthers blocked (PA-only), or pollinating anthers blocked (FA-only). New flowers were collected for each trial. To control for the potential behavioural response of bees to glue, each of the non-blocked anthers in all treatments also received a small amount of glue on the side of the anther. Because the four feeding anthers produce approximately 50% of a flower's total pollen (see *Results*), FA-only and PA-only flowers initially presented similar amounts of accessible pollen.

We attached each flower to the tip of 20-cm tall wire with a binder clip and placed this on a short (~50 cm) stool to form an artificial 1-flowered plant. Artificial plants were placed randomly in a 3x3 square grid with 1 m between plants. We then allowed a single bee to forage on the array for approximately 30 flower visits (7-26 minutes) and recorded the number and durations of visits to individual flowers using a tape recorder. This procedure was repeated using 22 different bees.

We employed ANOVA (*glm* package and Tukey tests (*multcomp* package) of log-transformed responses to assess differences among treatments. Analysis of visit number considered the total number of visits per treatment per trial (n = 66; 22 trials x 3 treatments). To analyze visit length, we first calculated the mean visit duration for each plant and then averaged these means for all plants in a given treatment and trial (n = 66).

To investigate whether pollinators preferentially manipulate feeding or pollinating anthers, we recorded visits by bees to Control, FA-only, or PA-only flowers for approximately 2 min with a digital video camera, and subsequently scored and analyzed behaviour using JWatcher Video ver. 1.0 (Blumstein & Daniel, 2007). We recorded 82 2-min foraging periods by 35 bees and we tried to have each bee experience all the three treatments although this was not always possible for every foraging run. We divided total visit duration into three categories: 1) flower handling—time spent not manipulating anthers, 2) anther buzzing—which occurred when a bee grasped either the pollinating or the feeding anthers with its mandibles and vibrated anthers to remove pollen (scored separately for each anther type), 3) grooming—active removal of pollen from the bee's body.

We analyzed the effect of bee identity and treatment on the duration (square-root transformed) of different behaviours using MANOVA. In this analysis, each of the behaviors was treated as the dependent variable. To determine whether blocking access to pollen of feeding anthers reduced the time spent collecting pollen, we also analyzed the effect of treatment (Control, FA-only, and PA-only) on the proportion of total visit duration spent buzzing the feeding anthers (arcsine transformed).

Pollen export efficiency

To determine whether pollen grains from feeding and pollinating anthers differ in their probability of reaching stigmas of other flowers, we compared pollen export from Control, FA-only, and PA-only flowers to recipient flowers with no accessible pollen. For each trial (21 total), we collected six flowers of similar size and randomly assigned half of them into one of the three donor treatments and the remainder to recipients (all anthers glued). We mounted flowers as described above and presented them individually, inside a 1.1x1.1x1.5 m flight cage, to *B. impatiens* workers that had previously been trained with unmanipulated flowers. Training flowers were removed from the cage 12 hours before each experimental trial to allow bees to groom themselves clean. Within each trial, the three donor treatments were randomly presented to the bees. After a flower was visited and buzzed, it was replaced by a recipient flower, which we allowed the bee to visit only once. We then excised the stigma of the recipient flower, placed it on a clean slide, stained it with fuchsine-stained glycerol jelly (Beattie, 1971), and gently squashed it under a cover slip. We then stored slides in a refrigerator until pollen grains were counted using a light microscope at 400 x magnification. Two independent observers counted each slide and we used the mean of the two counts in analyses (correlation coefficient between the two counts: ρ=0.972).

To assess pollen removal from donor flowers, we first estimated initial pollen availability. We counted the pollen grains from each anther in five flowers from each of 23 plants with an Elzone 282PC electronic particle counter (Particle Data, Inc., currently Micromeritics, Norcross, Georgia: see Harder, 1990b) and measured anther length and width (at the base) to calculate volume. To characterize the size range of electronically measured particles that should be counted as pollen, we measured pollen diameter microscopically (400x magnification) for two anthers from two randomly sampled flowers per accession, with 200 grains per sample. Pollen within ± 3 s.d. of the mean (i.e., >99.7% of the size distribution) fell between 13.75 and 25.04 μm diameter. We analyzed these data with a general linear model that considered anther volume (log[anther length * anther width^2]) and type (pollinating or feeding anther) as fixed effects, and accession, individual, and flower as random effects. We used this relation to estimate pollen availability in flowers by subtracting the count of pollen left in anthers after a bee visit from pollen availability estimated from anther measurements.

We tested the effect of treatment on pollen deposition using ANCOVA fitted via maximum likelihood (g*lm* package). Pollen deposition was square-root transformed prior to analysis to improve normality of the residuals. Models initially included treatment, pollen removal and buzz duration as explanatory variables, but buzz duration did not contribute significantly to variation in pollen deposition and was excluded. We compared treatments using Tukey's multiple comparison test (*multcomp* package).

Pollen placement and pollen grooming

Our observations indicated that pollinating and feeding anthers contact different areas of the pollinator's body (and see Jesson and Barrett, 2005). Because differential pollen placement may represent a potential mechanism to enforce division of labour (see Discussion) we conducted an experiment to characterize pollen placement by the two anther types. We allowed individual bees a single visit to either PA-only or FA-only flowers, freeze-killed them immediately after visitation and scored pollen deposition patterns. We visually scored the areas of the body (Dorsal, Ventral, Lateral) where pollen was deposited, as well as the density of the pollen using a four-color scale on a diagram of the bee's body, and analyzed the scanned images using digital image software (Adobe Photoshop CS2 ver. 9.0.2). We multiplied the area of the body covered by a density factor (high density = 1, medium density =0.666, low density = 0.333 and calculated the total pollen deposited in each area as the sum of (area covered)*(pollen density).

Bees actively engage in stereotypic behaviours such as pollen grooming. However, bees are not capable of grooming pollen from all areas of their body equally (see Kimsey, 1984). Because feeding and pollinating anthers may deposit pollen in different areas of a bee’s body, we investigated the ability of bumble bees to groom pollen from different parts of their bodies. We extracted pollen from *S. rostratum* flowers and applied it to anesthetized bees in a uniform layer using a paintbrush. We placed pollen-covered bees in a flight cage, and allowed them to groom for 15 min (n=10 bees), and then freeze-killed and pinned them. Two observers visually scored pollen coverage on each bee with a dissecting microscope using a four-point relative scale and we used the results to make a composite (“average”) image. We compared mean pollen density of experimental bees to the mean density observed on control bees (n=5), which were immediately freeze-killed after applying pollen.

Results

Pollen production of anther types

Pollen number per anther and pollen diameter differed significantly between feeding and pollinating anthers ($F_{1,438}$ = 2847.42, $F_{1,438}$ = 46.27, respectively; P < 0.01; Table 1), but did not differ between right- and left-handed flowers ($F_{1,89}$ = 2.97, $F_{1,89}$ = 0.003, respectively; P > 0.05). The pollinating anther in a flower produced 3.79 times more pollen than single feeding anthers (Table 1). Because each flower has four feeding anthers, the single pollinating anther produces on average 49% of the total pollen per flower. Pollen size differed by only ~0.1 µm between anther types (Table 1).

Pollinator behaviour on feeding and pollinating anthers

Aspects of pollinator visitation were influenced by accessibility of pollen from different anther types. As predicted, bees visited Control, FA-only, and PA-only flowers with equivalent frequency ($F_{2,63} = 0.65$, $P = 0.52$), suggesting that they did not discriminate among treatments when deciding which flowers to visit or revisit. In contrast, the mean duration of flower visits differed among treatments ($F_{2,63} = 3.66$, $P < 0.05$), because of briefer visits to PA-only flowers than to Control flowers (Tukey's tests, $P < 0.05$), with visit duration to FA-only flowers lying between these extremes.

The difference in pollinator behaviour on feeding and pollinating anthers was most evident through an examination of tasks executed by bees while on flowers. While visiting Control flowers, bees spent an average of 13.5% of their time handling (mean ± s.e. duration = 23.0 ± 2.8 s), 16.7% buzzing (28.1 ± 1.7 s), and 68.2% grooming (56.7±3.4 s). On Control flowers, 94.8% of buzzing time was directed to feeding anthers (26.3±1.7 seconds). The fraction of buzzing time directed to feeding anthers decreased significantly when access to pollen in the feeding anthers was blocked (PA-only treatment; 85.0 ± 2.7% of total buzzing time) relative to that for both the Control and FA-only treatments ($F_{2,79} = 10.46$, $P < 0.001$; Tukey test, $P < 0.05$), which did not differ (94.7 ± 1.9% and 97.1 ± 1.2% respectively; Tukey test, $P > 0.05$).

Pollen export of pollinating and feeding anthers

The effectiveness of feeding and pollinating anthers in exporting pollen to recipient stigmas was evaluated while controlling for the amount of pollen removed from donor flowers. Table 2A presents the mean number of pollen grains removed from donor flowers and the mean number of grains exported to recipient flowers. ANCOVA indicated that the partial regression coefficients of pollen export on pollen removal were equivalent for the three treatments (Treatment*Pollen removal interaction: $F_{2,56} = 1.46$, $P = 0.239$) and further analysis therefore excluded this interaction. Pollen export varied positively with pollen removal from the donor flower ($F_{1,58} = 4.03$, $P = 0.049$). As predicted, the number of pollen grains exported to stigmas of single recipient flowers differed significantly among treatments ($F_{1,58} = 3.24$, $P = 0.046$), with PA-only flowers exporting significantly more pollen than FA-only flowers (PA-only – FA-only = 14.04 ± 2.19 grains,), but as many pollen grains as Control flowers (PA-only – Control = 1.982 ± 1.784: Table 2B). Control flowers exported more pollen than FA-only flowers, on average, but this difference was not statistically significant (Control – FA-only = 5.47 ±1.946).

Pollen placement and grooming patterns

The analysis of pollen placement by FA-only and PA-only flowers indicated that feeding anthers deposited proportionally more pollen on the ventral surface of the bee than pollinating anthers ($F_{1,68} = 17.43$, $P < 0.001$), whereas pollinating anthers deposited more pollen on both the dorsal and the lateral surfaces of the bees ($F_{1,62} = 4.51$, $P < 0.05$, and $F_{1,62} = 4.21$, $P < 0.05$, respectively). In addition, the ability of pollinators to groom pollen was not uniform across their bodies. Visual inspection of the composite image of pollen remaining after 15min of grooming versus the control image (0min grooming) indicated that *B. impatiens* was less efficient at removing pollen from much of the bee's lower dorsal side, the mid and hind legs, and along the posterior lateral sides of the abdomen (data not shown). There were additional areas of high pollen density near the dorsal side of the bee's petiole (where the thorax and abdomen join), and along the notal midline. In contrast, little pollen usually remained on the ventral surface.

Theoretical analysis of heteranthery evolution

Our experimental results for *Solanum rostratum* support Müller's (1883) original proposal that heteranthery involves a division of labour between anthers into feeding and pollinating functions. Localized deposition of pollen on different parts of the bee's body and differential grooming of these pollen grains provide a mechanism to realize these different functions. We now present a phenotypic model that explores the consequences of differential grooming of pollen from feeding and pollinating anthers for the evolution and maintenance of heteranthery. The model incorporates features of the pollination process in plant species that are visited by pollen-consuming insects and have the potential to allocate resources to two anther types.

Model

We model pollination by considering separate pollen pools for feeding and pollinating anthers and allowing pollinator visitation to vary with pollen rewards (Fig. 1). Consider a population of hermaphroditic plants in which the resources available for stamen production (S) are allocated to stamens with pollinating and feeding anthers in proportions p and (1-p), respectively. Pollen production equals the product of resource availability divided by the cost per pollen grain (c). For simplicity, we assume that the fixed costs of making an anther are deducted from a separate resource pool. The number of pollen grains produced is then simply $N = S / c$.

We distinguish two multiplicative components of pollen export: the number of pollen grains exported per unit of visitation, *E*; and the number and duration of visits that a plant receives, collectively denoted by *h* (e.g. time spent visiting a plant or total number of visits). During visitation, pollinators remove a fraction of the pollen produced by the feeding anthers, of which a subsequent fraction is deposited on the pollinator's body, the product of which is represented by π, and the remaining pollen is lost from the pollination process. Of the pollen on a pollinator's body, proportion γ_f is collected as a reward by the pollinator (hereafter referred to as groomed) and the remainder, $1 - \gamma_f$, is available for pollination. A fraction θ of the non-groomed pollen is transferred to other plants' stigmas, whereas the remainder is lost. The same events occur for pollinating anthers with independent grooming probabilities, indicated by subscript *p*. Here, we restrict analysis to the simplest, and perhaps most interesting case, in which pollinators groom more pollen from feeding anthers than from pollinating anthers, i.e. $\gamma_f > \gamma_p$ (Fig. 1). This is likely to be particularly important for functional dimorphism, because for each anther type an increase in the grooming fraction (γ) jointly increases pollinator attraction and reduces pollen donation. In this case, pollen exported per unit of visitation (*E*) is:

$$E = k(p(1-\gamma_p) + (1-p)(1-\gamma_f)), \qquad (1)$$

where $k = \frac{S}{c}\pi\theta$.

We assume that pollinator visitation (*h*) varies as a power function of the proportion of pollen that a pollinator collects (Harder, 1990a; Rasheed & Harder, 1997ba), γ^λ, where $\lambda = 0$ when visitation varies independently of groomed pollen, $0 < \lambda < 1$ if visitation is an increasing, decelerating function of groomed pollen and $\lambda = 1$ if visitation increases linearly with groomed pollen (by visitation we mean both the number and length of visits). If, in addition, visitation depends on the pollen availability of a visited plant relative to the average in the population (*T*) (cf. Biernaskie & Elle, 2007), visitation equals

$$h = b\frac{\frac{S}{c}\pi\left(p\gamma_p^{\lambda} + (1-p)\gamma_f^{\lambda}\right)}{T}, \qquad (2)$$

where $T = \frac{S}{c}\pi\left(p\gamma_p^{\lambda} + (1-p)\gamma_f^{\lambda}\right)$ is averaged across all individuals in the population, and b is a scaling constant translating rewards to units of time spent in visitation. At equilibrium the mean visitation is simply $h = b$.

Evolutionary Dynamics

We now explore the evolutionary dynamics of a population in which a rare mutant that allocates a fraction p_m of resources to pollinating anthers arises, where the subscript *m* indicates mutant values. Because we seek the minimum conditions for division of labour to favour maintenance of heteranthery, we focus on the effects of stamen allocation on pollen export (outcross male fitness) relative to the average of other plants in the population (cf. Lloyd, 1992b; de Jong & Klinkhamer, 2005),

$$W_m = \frac{h_m E_m}{\bar{h}\bar{E}}\bar{x}, \qquad (3)$$

where *x* is the number of ovules available for cross-fertilization. Substitution of equations (1) and (2) into equation (3) yields:

$$W_m = \frac{\frac{S}{c}\pi\left(p_m\gamma_p^{\lambda} + (1-p_m)\gamma_f^{\lambda}\right)}{T}\frac{p_m(1-\gamma_p) + (1-p_m)(1-\gamma_f)}{p(1-\gamma_p) + (1-p)(1-\gamma_f)}\bar{x}, \qquad (4)$$

where $T = \frac{S}{c}\pi\left(p\gamma_p^\lambda + (1-p)\gamma_f^\lambda\right)$ is the average perceived reward for resident plants.

To determine the ESS for allocation to pollinating anthers ($\hat{p}$) we set $p_m = p = \hat{p}$, obtain the first derivative with respect to $\hat{p}$ and set this equation to zero (Otto & Day, 2007). After solving for $\hat{p}$, we find that the ESS allocation to pollinating anthers is

$$\hat{p} = \frac{1}{2}\left(\frac{\gamma_f^\lambda}{\gamma_f^\lambda - \gamma_p^\lambda} + \frac{(\gamma_f - 1)}{\gamma_f - \gamma_p}\right). \qquad (5)$$

The ESS allocation to pollinating anthers depends on three parameters: the fractions of groomed pollen (γ_f, γ_p), and the ability of pollinators to assess rewards (λ). Because $\gamma_f > \gamma_p$, by definition the first term in parenthesis in Eq. (5) is always positive, whereas the second term is always negative. The effects of the parameters on the ESS allocation to pollinating anthers are difficult to interpret by inspection.

We begin by addressing whether heteranthery (i.e., $0 < \hat{p} < 1$) can invade a population with just one anther type. It can be shown that in the case of a single type of anther, and thus a single pollen pool, equation (4) can be written as:

$$W_m = \frac{\gamma_m^\lambda (1-\gamma_m)}{\gamma^\lambda (1-\gamma)}\overline{x},$$

where the subscript *m* indicates the fraction of groomed pollen in the mutant introduced at low frequency in the population. Applying an ESS analysis as above yields the optimal fraction of pollen that the plant should offer to the pollinators for grooming:

$$\hat{\gamma} = \frac{\lambda}{1+\lambda}. \qquad (6)$$

When pollinators do not adjust visitation based on groomed rewards $(\lambda = 0)$ plants do not benefit from allowing pollinators to groom any pollen, i.e. $\hat{\gamma} = 0$. In contrast, when pollinators adjust visitation to groomed rewards, plants should allow pollinators to groom some pollen in exchange for pollinator services.

We investigated whether heteranthery is an evolutionarily stable strategy in a population in which, from the plant's perspective, pollinators groom the optimal amount of pollen from the feeding anthers, $\hat{\gamma}_f = \hat{\gamma}$. In other words, we investigate whether a plant should invest in specialized pollinating anthers, given that feeding anthers are providing the right amount of pollen in exchange for visitation services. Numerical analyses were conducted by substituting a wide range of parameter values for γ_p and λ, and determining whether heteranthery (i.e. values of $\hat{p}$ between 0 and 1) could invade. Our results (not shown) indicate that when $\gamma_f = \hat{\gamma}_f$, a plant with an anther that contributes less to the groomed pollen pool (pollinating anther) cannot successfully invade and be maintained in a resident population with uniform anthers, and thus heteranthery does not evolve.

Now, consider the case when pollinators groom more than the optimal amount of pollen from the feeding anthers, $\gamma_f > \hat{\gamma}_f$, so they act as pollen consumers beyond the interests of the plant. Contrary to the previous case, heteranthery is an ESS under a wide range of conditions. Figure 3 shows the ESS allocation to pollinating anthers as a function of the fraction of pollen groomed from the pollinating anthers (γ_p) when pollinators groom 20%, 60% and 100% more pollen from the feeding anthers than the optimal value for the uniform anther condition, and λ = 1. Figure 3 illustrates that heteranthery is evolutionarily stable when pollinating anthers allow pollinators to groom a fraction of pollen smaller than $\hat{\gamma}_f$. Moreover, under conditions that allow heteranthery to evolve, increased grooming of feeding anther pollen (e.g. more grains packed into corbiculae) promotes increased allocation to pollinating anthers (Fig. 3).

When $\lambda = 1$, heteranthery is always an ESS and the optimal allocation to pollinating anthers is a decreasing function of γ_p. This is because of the negative effect on pollinator attraction of diminishing groomed rewards from the pollinating anthers (Fig. 3). Significantly, as pollinators become less able to adjust the visitation response to groomed rewards (smaller λ), heteranthery is favoured only at high values of γ_p (relative to γ_f; Fig. 4). For example, when $\lambda = 0.5$ $(\gamma_f = 0.39)$, $\gamma_p < 0.2$ favours flowers with only feeding anthers (Fig. 4).

Discussion

Our results support Müller's hypothesis (1883) that anther dimorphism in heterantherous flowers involves a division of labour and represents a floral strategy for coping with pollinators that consume pollen. *Bombus impatiens* visiting *Solanum rostratum* flowers found feeding anthers more attractive than pollinating anthers. They spent more time extracting pollen from feeding anthers and responded to blocked feeding anthers by reduced foraging on flowers. The contrasting attention to the two anther types contributed towards pollinating anthers dispersing proportionately more pollen than feeding anthers (Table 2B). Our ESS analysis of the pollination process revealed that anther dimorphism is favoured when pollen-collecting insects remove more pollen than the optimum that plants should exchange for visitation services. Accordingly, increased pollen consumption from feeding anthers promotes more allocation of resources to pollen in pollinating anthers (Fig. 2). A pollinator's ability to adjust its visitation to the amount of pollen collected influences whether heteranthery will be selectively maintained. The better pollinators are at adjusting their visitation to the amount of pollen groomed the more likely it is that heteranthery will evolve (Fig. 3).

Anther dimorphism and division of labour

Functional differentiation between pollinating and feeding anthers: pollinator behaviour

Previous studies of heterantherous species have found that the removal of feeding anthers (e.g. Tang & Huang, 2007), as well as the removal of pollinating anthers while blocking access to pollen in feeding anthers (Luo et al., 2008a), both reduce pollinator visitation. Because these manipulations removed not only the advertising structures (anthers) but also the reward (pollen) these experimental designs do not identify whether bees can actively respond to differences in pollen availability independent of changes in advertising structures. Our results show such a response: pollinators spend less time at intact but nonfunctional feeding anthers, as if they perceive them as unrewarding. In contrast, bees did not show shorter visitation to flowers whose pollinating anthers had been rendered unrewarding. The implication of these results is that bees modify their foraging behaviour in response to the amount of pollen extracted from feeding anthers only. Coupled with the disproportionate fraction of time bees devoted to feeding anthers over pollinating anthers in Control flowers, the bees' responses are concordant with the specialization of feeding anthers into attractive functions.

Why are pollinating anthers less attractive to pollinators than feeding anthers? Plants visited by pollen-collecting insects often use both pollen and anthers as attractant signals (Lunau, 2000). In many buzz-pollinated species, anthers assume a signalling function by virtue of being of a similar colour to pollen and reflecting comparable UV patterns (Lunau, 2000, 2007). In many heterantherous species, pollinating anthers are differently coloured from feeding anthers. For example, feeding anthers are often yellow, while the pollinating anther is either the same colour as the petals and hence cryptic (e.g. *Solanum citrullifolium*, many species of Melastomataceae, and *Heteranthera* and *Monochoria* of Pontederiaceae), or a different, possibly less attractive colour distinct from pollen (e.g. reddish-brown in *Solanum rostratum* and *S. grayi*). Colour dimorphism of anthers therefore seems likely to play an important role in governing pollinator preferences for the two anther types.

Differences in attractiveness after pollinators have landed on flowers, as detected in our study, could also arise if pollen from different anther types differs in odour, as bees use olfactory cues released by pollen during visitation (Dobson & Bergstrom, 2000). Another possibility is that the reduced attractiveness of pollinating anthers occurs because bees have more difficulty manipulating them than feeding anthers. For example, medium to large bees usually hold feeding anthers with their mandibles while vibrating flowers and they collect pollen from the ventral surface of their abdomens (Buchmann, 1983). In *S. rostratum* and many other heterantherous species the deflected position of the pollinating anther away from the feeding anthers probably makes it difficult for bees to position themselves effectively for pollen collection. This may increase the effort required to extract pollen. This problem may be reduced for smaller bees which may be able to land directly on pollinating anthers (Gross & Kukuk, 2001); however, small bees are often unable to efficiently extract pollen by buzzing (see Snow & Roubik, 1987).

Functional differentiation between pollinating and feeding anthers: pollen export

Studies of pollen analogues have found mixed evidence for the ability of pollinating anthers to export pollen more effectively than feeding anthers (Bowers, 1975; Wolfe et al., 1991; Wolfe & Estes, 1992). Taking advantage of a difference in exine sculpture between pollen of feeding and pollinating anthers in *M. malabatrichum*, Luo et al. (2008a) demonstrated that stigmas receive a higher proportion of pollen from pollinating anthers than feeding anthers after a single carpenter bee visit. However, previous studies that have estimated pollen transfer have failed to account for differences between anther types in both the amount of pollen available for export and the amount of pollen removed during visitation (e.g. Tang & Huang, 2007; Luo et al., 2008a). As a result, it has not been possible to evaluate whether pollinating anthers have a disproportionate ability to export pollen per grain removed. We demonstrated that PA-only flowers export as many pollen grains as control flowers and significantly more than FA-only flowers (Tables 2A and 2B). Because this analysis accounted for pollen removal from donor flowers (Table 2B), the result is consistent with the predictions of the division-of-labour hypothesis that pollen from pollinating anthers is more likely than pollen from feeding anthers to be exported to stigmas of other plants. Pollinating anthers probably export more pollen than feeding anthers because of their location within a flower. Pollen from pollinating anthers is deposited on a different area of the pollinator's body than pollen from feeding anthers and this likely affects the probability of stigma contact and the ability of bees to groom this pollen.

Our experiments on pollen placement indicate that pollinating anthers deposit more pollen on the dorsal and lateral surfaces of the bee, while feeding anthers deposit more pollen on the ventral surface. Moreover, our observations of captive bees indicate that *B. impatiens* is less efficient at grooming pollen from the lower dorsal side, and upper lateral sides of the abdomen than from the ventral side of the abdomen. Jesson and Barrett (2005) found that individuals of *B. impatiens* contact *S. rostratum* stigmas mostly on the lateral (50% including pollen baskets) and dorsal surfaces of the abdomen (17%), and only rarely on the ventral surface (7%). Thus, effective pollen transfer by the pollinating anther is probably favoured by a more precise correspondence between pollen placement and stigma contact and a reduced ability of the pollinator to groom pollen deposited by these anthers.

Functional differentiation between pollinating and feeding anthers: pollen viability

Specialization of anther functions may also be accompanied by variation in pollen traits, including viability (Buchmann, 1983). For example, in *Commelina coelestis* and *C. dianthifolia*, some feeding anthers produce pollen grains of low viability (Müller, 1882; Hrycan & Davis, 2005). Sterility of pollen from feeding anthers has also been reported in *Tripogandra*, another member of the Commelinaceae (Mattson, 1976). Similarly, Forbes (1882) reports differences in pollen size, shape, and fertility between feeding and pollinating anthers in species of Melastomataceae. In these cases, feeding anther pollen is large, three-cornered and apparently sterile, whereas pollen from pollinating anthers is smaller, oval-shaped and fertile. Higher proportions of viable pollen grains in long ("pollinating") anthers than in mid and short anthers ("feeding") have also been found in some species of *Senna* (*S. alata* and *S. bicapsularis*; Fabaceae) but not in others (*S. surattensis*) (Luo et al., 2008b). There is a size difference between pollen of pollinating and feeding anthers in *Solanum rostratum,* but it is trivial (0.1µm or approximately 0.5% of grain diameter), and any differences in pollen germination, viability, or the ability of pollen to sire ovules are also negligible (Bowers, 1975). Heteranthery thus involves a continuum of anther specialization and pollen function (Lloyd 2000). This can range from the involvement of largely sterile structures, as in some species of Commelinaceae, to species like *Solanum rostratum* in which pollen from both anther types has the potential to participate in cross-fertilization.

The evolution of heteranthery as a strategy to reconcile the dual fates of pollen

Our model predicts that heteranthery can evolve when anther dimorphism causes differences in the probability of pollen grooming between anther types. Because groomed pollen serves as a reward (Vogel, 1978; Buchmann, 1983) but reduces pollen available for export (Harder, 1990a), anther traits that promote a higher probability of pollen grooming are more attractive to pollinators and, accordingly, are less successful in contributing pollen for cross-pollination. This tradeoff favours division of labour, with one set of anthers serving to reward pollinators (feeding anthers) and the other primarily involved in pollen export (pollinating anthers). Our model demonstrates that heteranthery evolves only when pollinators consume more pollen than a plant should optimally provide in exchange for pollination services (Fig. 2). When heteranthery evolves, enhanced pollen consumption from feeding anthers favours increased allocation to pollinating anthers (Fig. 2), so changes in the relative amount of pollen groomed from feeding and pollinating anthers represent functional differentiation between anther types. Implementation of functional differentiation between stamens seems likely to be constrained by both plant and pollinator characteristics and this may explain why heteranthery is not more widespread in the flowering plants.

The evolution and maintenance of heteranthery depends on pollinators acting as “smart consumers”, as it evolves more readily when pollinators can assess the amount of pollen they groom (Fig. 3). The shorter visits in response to blocked feeding anthers indicate that bumble bees respond to changes in pollen availability and presentation (e.g. Buchmann & Cane, 1989; Harder, 1990a; Rasheed & Harder, 1997a, b; Luo et al., 2008a). Determination of the extent of pollen consumption by pollinators and whether they adjust visitation to rewards groomed in different species of nectarless flowers should help establish how often the conditions favouring heteranthery occur

Fitness through male reproductive function is reduced when pollinators consume pollen that could otherwise cross-fertilize ovules. This situation is especially severe in species in which pollen is the only reward (i.e. pollen flowers), as in most heterantherous taxa. This problem can be reduced in at least five non-exclusive ways (see Vogel, 1978; Harder, 1990a): (1) Using only pollen that would otherwise be lost during the pollination process (e.g. falling to the ground) as a reward (Harder & Wilson, 1997); (2) Limiting pollen access in all anthers either by restricting pollen removal via pollen packaging and dispensing strategies (Harder & Thomson, 1989), or by placing pollen on pollinators where it is difficult to groom (Brantjes, 1982; Macior, 1982; Kimsey, 1984); (3) Production of copious small pollen grains (Buchmann, 1983); (4) Pollinator attraction without providing rewards (e.g. deceit pollination, Schemske & Ågren, 1995), or attraction using non-rewarding structures like staminodes (Lunau, 2000; Walker-Larsen & Harder, 2000); and (5) Functional division of labour—specialization—of pollen types into attraction and fertilization functions (e.g. Vogel, 1978). These diverse ways of reconciling the dual function of pollen in nectarless species represent the escalating refinement of strategies for dealing with pollen-consuming insects by minimizing the fitness costs associated with conflicting functions. Darwin’s enigma of the adaptive significance of heteranthery can therefore be explained as a floral mechanism that results in the functional specialization of pollen into fertilization and feeding functions.

Acknowledgements

We thank K. Ostevik, I. Blázquez-Paz, J. Brett, and several undergraduate students for technical assistance; C. Rueffler, J. Biernaskie, and C. Jordan for advice on the heteranthery model, and J. Stinchcombe for statistical help. Lawrence Harder provided insightful comments on an earlier version of the manuscript. MVM was supported by a post-doctoral fellowship from the Canada Research Chair's program to SCHB. JSM was supported by an NSERC-CGS scholarship. Funding for this work was supported by NSERC Discovery Grants to JDT and SCHB.

References

Beattie, A. J. 1971. A technique for the study of insect-borne pollen. *Pan-Pac. Entomol.* **47**: 82.

Biernaskie, J. M. & Elle, E. 2007. A theory for exaggerated secondary sexual traits in animal-pollinated plants. *Evol. Ecol.* **21**: 459-472.

Blumstein, D. T. & Daniel, J. C. 2007. *Quantifying Behavior the JWatcher Way*. Sinauer Associates, Inc., Sunderland, Massachusetts.

Bowers, K. A. W. 1975. The pollination ecology of *Solanum rostratum* (Solanaceae). *Am. J. Bot.* **62**: 633-638.

Brantjes, N. B. M. 1982. Pollen placement and reproductive isolation between two Brazilian *Polygala* species (Polygalaceae). *Plant Syst. Evol.* **141**: 41-52.

Buchmann, S. L. 1983. Buzz pollination in angiosperms. In: *Handbook of Experimental Pollination Biology*, (Jones, C. E. & Little, R. J., eds.). pp. 73-113. Scientific and Academic Editions, NY.

Buchmann, S. L. & Cane, J. H. 1989. Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia* **81**: 289-294.

Darwin, C. 1877. *The Different Forms of Flowers on Plants of the Same Species*. John Murray, London.

Darwin, F. 1899. The botanical work of Darwin. *Ann.Bot.* **13**: ix-xix.

de Jong, T. J. & Klinkhamer, P. G. L. 2005. *Evolutionary Ecology of Plant Reproductive Strategies*. Cambridge University Press, Cambridge.

Dobson, H. E. M. & Bergstrom, G. 2000. The ecology and evolution of pollen odors. *Plant Syst. Evol.***222**: 63-87.

Dulberger, R. 1981. The floral biology of *Cassia didymobotrya* and *Cassia auriculata* (Caesalpiniaceae). *Am. J. Bot.* **68**: 1350-1360.

Endress, P. K. 1994. *Diversity and Evolutionary Biology of Tropical Flowers*. Cambridge Universtity Press, Cambdridge.

Endress, P. K. 1997. Relationships between floral organization, architecture, and pollination mode in *Dillenia* (Dilleniaceae). *Plant Syst. Evol.* **206**: 99-118.

Graham, S. W. & Barrett, S. C. H. 1995. Phylogenetic systematics of Pontederiales: Implications for breeding-system evolution. In: *Monocotyledons: Systematics and Evolution*, (Rudall, P. J., Cribb, P. J., Cutler, D. F. & Humphries, C. J., eds.). pp. 415-441. Royal Botanic Gardens, Kew.

Gross, C. L. & Kukuk, P. F. 2001. Foraging strategies of *Amegilla anomola* at the flowers of *Melastoma affine*: No evidence for separate feeding and pollinating anthers. *Acta Hort.* **561**: 171-178.

Harder, L. D. 1990a. Behavioral responses by bumble bees to variation in pollen availability. *Oecologia* **85**: 41-47.

Harder, L. D. 1990b. Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology* **71**: 1110-1125.

Harder, L. D. & Barrett, S. C. H. 1996. Pollen dispersal and mating patterns in animal-pollinated plants. In: *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants*, (Lloyd, D. G. & Barrett, S. C. H., eds.). pp. 140-190. Chapman and Hall, NY.

Harder, L. D. & Thomson, J. D. 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *Am. Nat.* **133**: 323-344.

Harder, L. D. & Wilson, W. G. 1997. Theoretical perspectives on pollination. *Acta Hort.* **437**: 83-101.

Harris, J. A. & Kuchs, O. M. 1902. Observations on the pollination of *Solanum rostratum* Dunal and *Cassia chamaecrista* L. *Kansas Univ. Sci. Bull.* **1**: 15-41.

Hrycan, W. C. & Davis, A. R. 2005. Comparative structure and pollen production of the stamens and pollinator-deceptive staminodes of *Commelina coelestis* and *C. dianthifolia* (Commelinaceae). *Ann. Bot.* **95**: 1113-1130.

Jesson, L. K. & Barrett, S. C. H. 2002. Enantiostyly: Solving the puzzle of mirror-image flowers. *Nature* **417**: 707-707.

Jesson, L. K. & Barrett, S. C. H. 2003. The comparative biology of mirror-image flowers. *Int. J. Plant Sci.* **164**: S237-S249.

Jesson, L. K. & Barrett, S. C. H. 2005. Experimental tests of the function of mirror-image flowers. *Biol. J. Linn. Soc.* **85**: 167-179.

Kimsey, L. S. 1984. The behavioral and structural aspects of grooming and related activities in euglossine bees (Hymenoptera, Apidae). *J. Zool.* **204**: 541-550.

Lester, R. N., Francisco-Ortega, J. & Al-Ani, M. 1999. Convergent evolution of heterandry (unequal stamens) in *Solanum*, proved by spermoderm SEM. In: *Solanaceae IV*, (Nee, M., Symon, D. E., Lester, R. N. & Jessop, J. P., eds.). pp. 51-69. Royal Botanic Gardens, Kew.

Levin, R. A., Myers, N. R. & Bohs, L. 2006. Phylogenetic relationships among the "spiny solanums" (*Solanum* subgenus *Leptostemonum*, Solanaceae). *Am. J. Bot.* **93**: 157-169.

Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* **113**: 67-79.

Lloyd, D. G. 1992a. Evolutionary stable strategies of reproduction in plants: who benefits and how? In: *Ecology and Evolution of Plant Reproduction*, (Wyatt, R., ed.). pp. 137-168. Chapman & Hall, NY.

Lloyd, D. G. 1992b. Self-fertilization and cross-fertilization in plants. II. The selection of self-fertilization. *Int. J. Plant Sci.* **153**: 370-380.

Lloyd, D. G. 2000. The selection of social actions in families: III. Reproductively disabled individuals and organs. *Evol. Ecol. Res.* **2**: 29-40.

Lunau, K. 2000. The ecology and evolution of visual pollen signals. *Plant Syst. Evol.* **222**: 89-111.

Lunau, K. 2007. Stamens and mimic stamens as components of floral colour patterns. *Bot. Jahrb. Syst.* **127**: 13-41.

Luo, Z., Zhang, D. & Renner, S. S. 2008a. Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labour hypothesis. *Func. Ecol.* **22**: 794-800.

Luo, Z.-L., Gu, L. & Zhang, D.-X. 2008b. Intrafloral differentiation of stamens in heterantherous flowers. *J. Syst. Evol.* **46**. doi: 10.3724/SP.J.1002.2008.08019.

Macior, L. W. 1982. Plant community and pollinator dynamics in the evolution of pollination mechanisms in *Pedicularis* (Scrophulariaceae). In: *Pollination and Evolution*, (Armstrong, J. A., Powell, J. M. & Richards, A. J., eds.). pp. 29-45. Royal Botanical Gardens, Sydney.

Marazzi, B., Conti, E. & Endress, P. K. 2007. Diversity in anthers and stigmas in the buzz-pollinated genus *Senna* (Leguminosae, Cassiinae). *Int. J. Plant Sci.* **168**: 371-391.

Mattson, O. 1976. The development of dimorphic pollen in *Tripogandra* (Commelinaceae). In: *The Evolutionary Significance of the Exine*, (Ferguson, I. K. & Muller, J., eds.). pp. 163-183. Academic Press, New York.

Maynard-Smith, J. 1982. *Evolution and the Theory of Games*. Cambridge University Press, Cambridge.

Morgan, M. T. 2006. Selection on reproductive characters: Conceptual foundations and their extension to pollinator interactions. In: *Ecology and Evolution of Flowers*, (Harder, L. D. & Barrett, S. C. H., eds.). pp. 25-40. Oxford University Press, Oxford.

Müller, F. 1883. Two kinds of stamens with different functions in the same flower. *Nature* **27**: 364-365.

Müller, H. 1881. Two kinds of stamens with different functions in the same flower. *Nature* **24**: 307-308.

Müller, H. 1882. Two kinds of stamens with different functions in the same flower. *Nature* **26**: 30.

Otto, S. P. & Day, T. 2007. *A Biologist's Guide to Mathematical Modelling in Ecology and Evolution*. Princeton University Press, Princeton.

R-Development Core Team 2008. R. A language and environment for statistical computing. pp. Foundation for Statistical Computing, Vienna.

Rasheed, S. A. & Harder, L. D. 1997a. Economic motivation for plant species preferences of pollen-collecting bumble bees. *Ecol. Entomol.* **22**: 209-219.

Rasheed, S. A. & Harder, L. D. 1997b. Foraging currencies for non-energetic resources: pollen collection by bumblebees. *Anim. Behav.* **54**: 911-926.

Schemske, D. W. & Ågren, J. 1995. Deceit pollination and selection on female flower size in *Begonia involucrata*. An experimental approach. *Evolution* **49**: 207-214.

Snow, A. A. & Roubik, D. W. 1987. Pollen deposition and removal by bees visiting two tree species in Panama. *Biotropica* **19**: 57-63.

Tang, L. L. & Huang, S. Q. 2007. Evidence for reductions in floral attractants with increased selfing rates in two heterandrous species. *New Phytol.* **175**: 588-595.

Todd, J. E. 1882. On the flowers of *Solanum rostratum* and *Cassia chamaecrista*. *Am. Nat.* **16**: 281-287.

Ushimaru, A., Watanabe, T. & Nakata, K. 2007. Colored floral organs influence pollinator behavior and pollen transfer in *Commelina communis* (Commelinaceae). *Am. J. Bot.* **94**: 249-258.

Vogel, S. 1978. Evolutionary shifts from reward to deception in pollen flowers. In: *The Pollination of Flowers by Insects*, (Richards, A. J., ed.). pp.89-96. Academic Press, London.

Walker-Larsen, J. & Harder, L. D. 2000. The evolution of staminodes in angiosperms: Patterns of stamen reduction, loss, and functional re-invention. *Am. J. Bot.* **87**: 1367-1384.

Whalen, M. D. 1979. Taxonomy of *Solanum* section *Androceras*. *Gentes Herbarum* **11**: 359-426.

Wolfe, A. D. & Estes, J. R. 1992. Pollination and the function of floral parts in *Chamaecrista fasciculata* (Fabaceae). *Am. J. Bot.* **79**: 314-317.

Wolfe, A. D., Estes, J. R. & Chissoe, W. F. 1991. Tracking pollen flow of *Solanum rostratum* (Solanaceae) using backscatter scanning electron microscopy and X-ray microanalysis. *Am. J. Bot.* **78**: 1503-1507.

Table 1. Summary of mean (SE) anther and pollen characteristics for feeding and pollinating anthers of *Solanum rostratum*. Anther volume was approximated as length*width^2.

Anther type	Anthers / flower	Volume per anther (mm^3)	Pollen number per anther	Total pollen per anther type	Pollen diameter (µm)	Sample size (# anthers)
Feeding Anthers	4	16.417 (0.201)	162×10^3 (2.03×10^3)	651×10^3	19.086 (0.014)	440
Pollinating Anthers	1	63.496 (1.372)	614×10^3 (17.40×10^3)	614×10^3	18.979 (0.028)	112
Per flower total	5	---	---	1264×10^3 (25.71×10^3)	19.064 (0.013)	552

Table 2. (A) Mean and 95% confidence intervals for number of pollen grains deposited on recipient flowers, and relative pollen removed from donor flowers by *Bombus impatiens* visiting paired donor and recipient flowers of *Solanum rostratum*. Experimental treatments were: Control, feeding anther only (FA-only), and pollinating anther only (PA-only). **(B)** Multiple comparisons of the difference in mean pollen (SE) exported from donor to recipient flowers of *Solanum rostratum*. The analysis was conducted in a model incorporating the estimated number of pollen grains removed from donor flowers as a covariate. *P*-values are adjusted to correct for multiple comparisons.

(A)

Treatment	Pollen on recipient stigma	Pollen removed from donor (in 10^3)	Number of trials
Control	92.48 (58.09 – 134.83)	33.05 (-94.80 – 161.41)	21
FA-only	68.14 (37.43 – 108.00)	241.07 (160.98 – 321.17)	21
PA-only	113.90 (78.74 – 155.55)	-40.67 (-145.30 – 63.96)	21

(B)

Comparison	Estimate	*z*-value	*P*-value
PA-only – FA-only	14.04 (2.19)	2.53	0.03
Control – FA-only	5.47 (1.95)	1.67	0.21
Control – PA-only	-1.98 (1.77)	-1.05	0.54

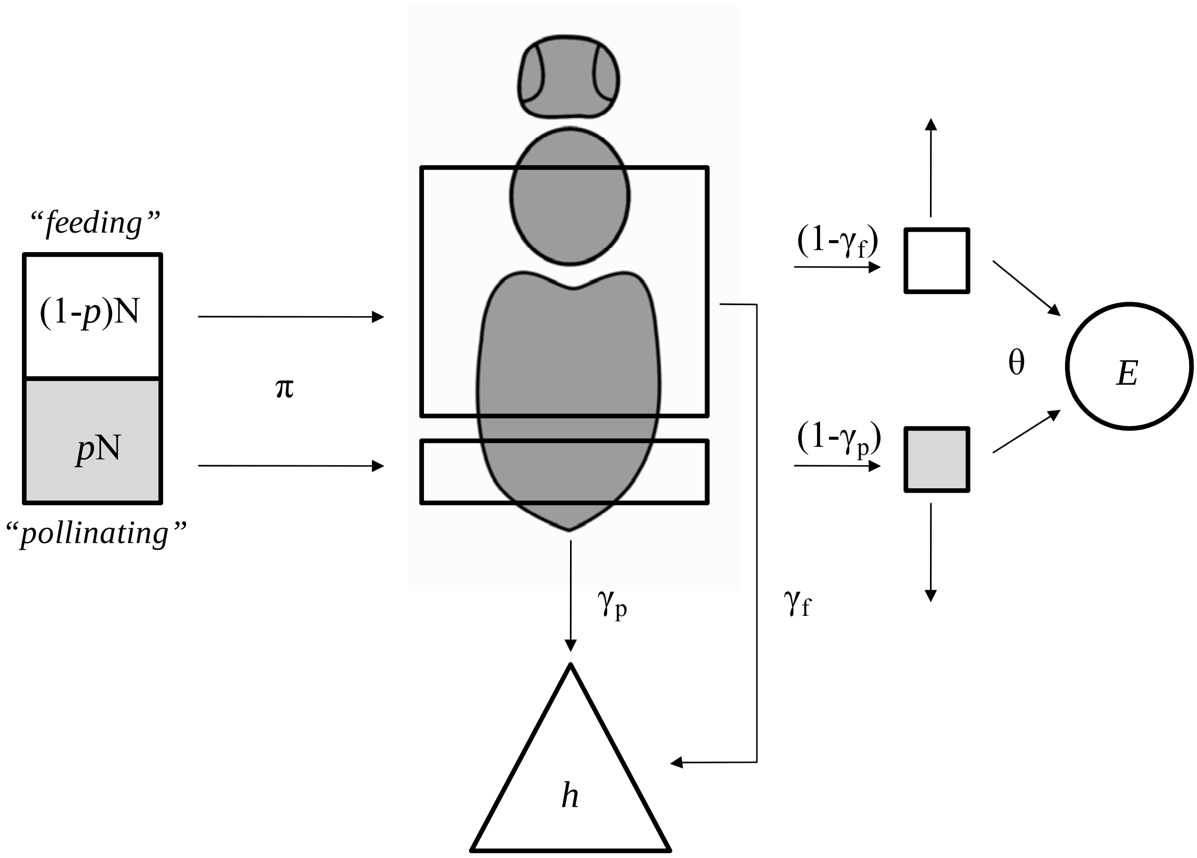

Figure 1. Graphical depiction of the pollination process in a species with heteranthery. This model is broadly based on similar compartment models in Harder and Barrett (1996) and Harder and Wilson (1998). In contrast with previous models, this model assumes that the collection (grooming) of pollen by pollinators increases visitation (h), and that pollen production is divided into pollinating and feeding anthers. Parameters as follows: p = allocation to pollinating anthers, n = pollen grains, π= fraction of pollen removed and deposited on the pollinator's body, γ = fraction of pollen consumed (groomed) by the pollinator and thus unavailable for ovule fertilization, θ = fraction of pollen transferred to the stigmas of other plants, h = visitation, E = number of pollen grains deposited on other stigmas.

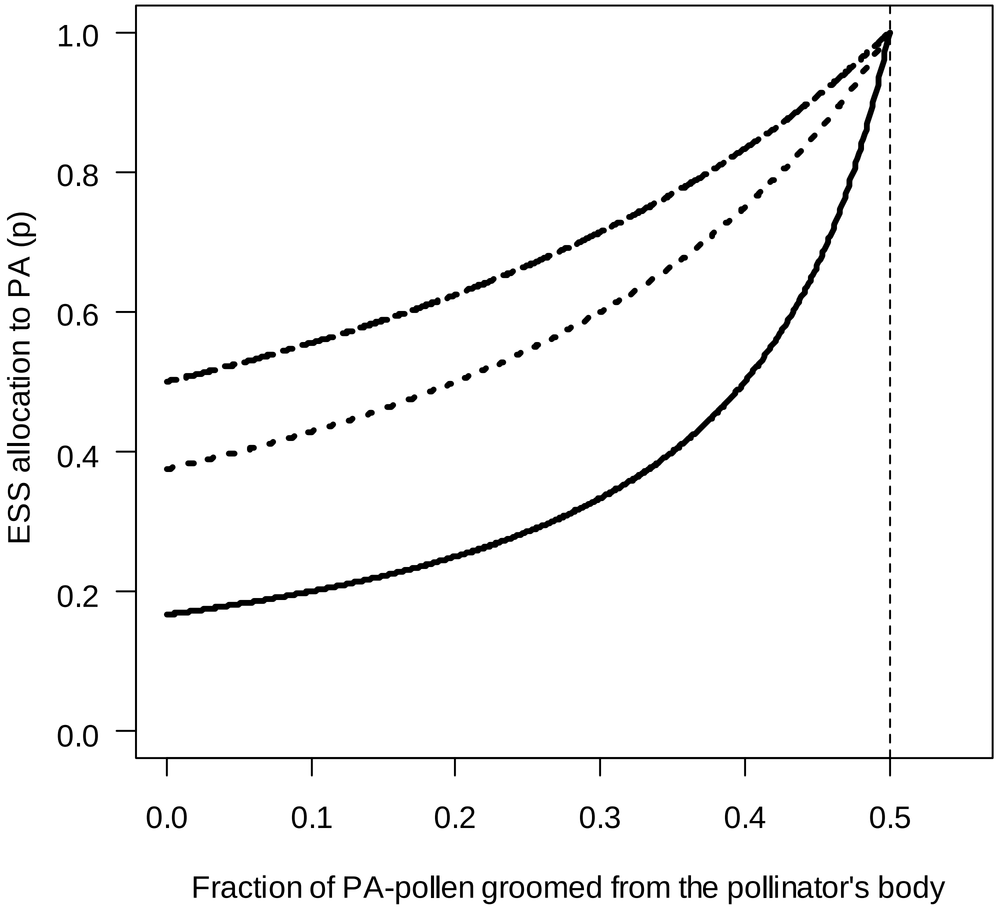

Figure 2. Evolutionarily stable allocation to pollinating anthers (PA) (*p*, proportion of stamen resources) as a function of the fraction of PA-pollen groomed from the pollinator's body (γ_p). Heteranthery occurs when $0 < p < 1$. The lines show the allocation to pollinating anthers for different parameter values of the amount of feeding anther pollen groomed. Solid line: $\gamma_f = 0.6$, dashed line: $\gamma_f = 0.8$, dot-dash line: $\gamma_f = 1$. The vertical line represents the optimal fraction of pollen groomed ($\hat{\gamma}_f = 0.5$), expected for plants with a single type of anther. For this figure, λ was set to one.

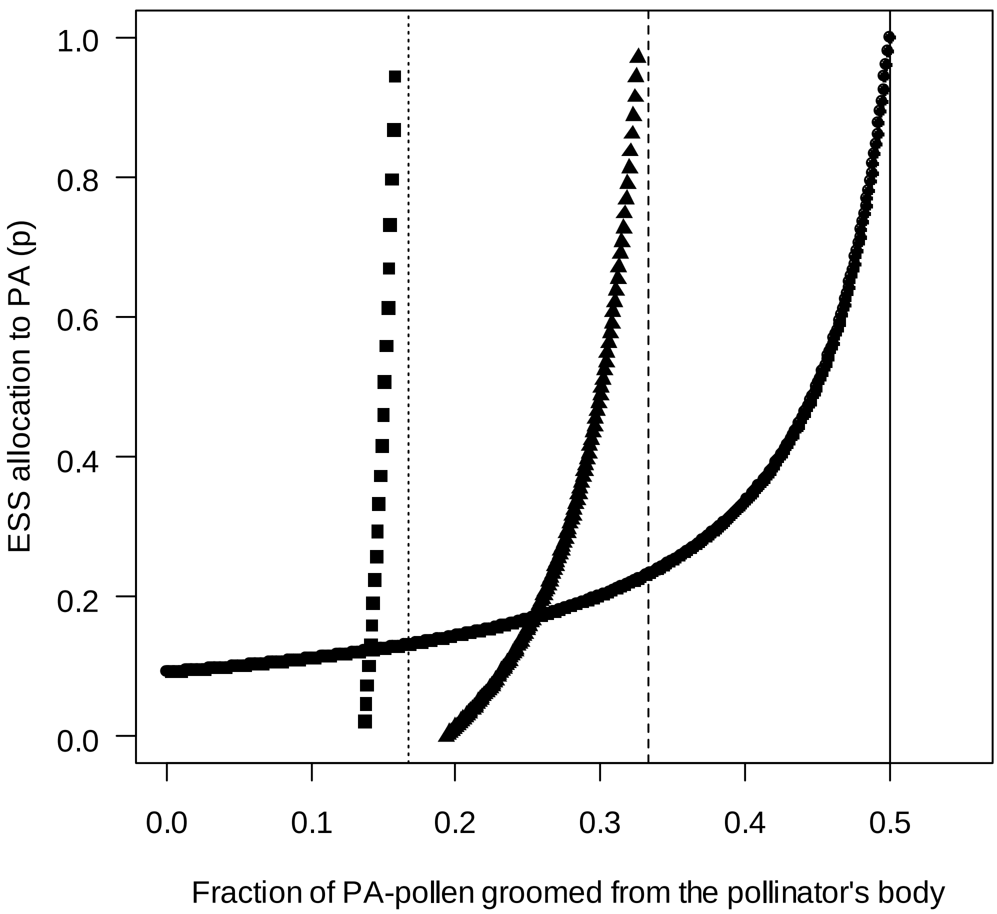

Figure 3. Evolutionarily stable allocation to pollinating anthers (PA) (*p*, proportion of stamen resources) as a function of the fraction of PA-pollen groomed from the pollinator's body (γ_p), for different values of the ability of pollinators to adjust visitation to the amount of rewards groomed from their body (λ). Circles: λ = 1, triangles: λ = 0.5, squares: λ = 0.2. The fraction of FA-pollen groomed (γ_f) was set to 110% of the optimal value for the case of plants with uniform anthers ($\gamma_f = 1.1\hat{\gamma}_f$), which is indicated with vertical lines.