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# Reproduction in Flame Azalea (*Rhododendron calendulaceum*, Ericaceae): A Rare Case of Insect Wing Pollination

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abstract: Although many angiosperms are serviced by flying pollinators, reports of wings as pollen vectors are rare. Flame azalea (*Rhododendron calendulaceum*) is visited by diverse insects, yet previous observations suggested that only butterfly wings may transfer pollen to stigmas. We used an experimental approach to determine whether butterfly wings are the primary vehicle of pollination in flame azalea. Over two seasons of observations, only butterflies (*Papilio glaucus* and *Speyeria cybele*) contacted both anthers and stigmas, yet because of differences in wing-flapping behavior, *P. glaucus* transferred pollen most efficiently. In contrast, bee species specialized either on pollen or nectar but did not contact both anthers and stigmas. A field experiment revealed that flowers excluding butterflies experienced almost complete fruit failure, whereas fruit set in open flowers did not differ from those that were hand pollinated. Additionally, butterflies had 56-fold more azalea pollen on their wings than bodies, while azalea stigmas bore both pollen and wing scales. These results suggest that plants with many visitors contacting reproductive organs may still specialize on a single guild of visitors for pollination and that wing-borne pollen transfer is a key mode of flame azalea pollination.

Keywords: flame azalea, *Rhododendron calendulaceum*, specialization, butterfly wing, pollination, floral biology.

## Introduction

The majority of flowering plants require animal vectors to transport pollen for reproductive success. Indeed, we see an array of adaptations and behaviors in both plants and their pollinators as a result of selection for animal feeding and effective pollen dispersal (Barrett 2010). Such variations have led to the concept of pollination syndromes, whereby species that are serviced by the same guild of pollinators tend to converge on similar floral displays with re-

spect to flower color, size, reward, and inflorescence structure (Stebbins 1970; Faegri and van der Pijl 1979; Fenster et al. 2004; Wolfe and Sowell 2006). The pollination syndrome hypothesis necessitates a high level of specialization in plant-pollinator relationships (Johnson and Steiner 2000). However, the literature is filled with studies showing that most plant species are visited by diverse pollinator communities, suggesting a greater level of generalization (Waser et al. 1996; Rosas-Guerrero et al. 2014). A key challenge presented to pollination biologists is to determine objectively which of the various flower visitors are actually effective pollinators (Schemske and Horvitz 1984; Pellmyr and Thompson 1996; Kay and Schemske 2003).

Flame azalea, *Rhododendron calendulaceum* (Michx.) Torr. (Ericaceae), is a common nonclonal understory shrub of eastern deciduous forests (Gleason and Cronquist 1963). Plants produce large and showy yellow-orange flowers that can remain in anthesis for up to 14 days (Blair and Wolfe 2007). While a variety of insects visit the flowers of *R. calendulaceum*, field observations suggest that only the wings of large butterflies make contact with both male and female flower parts (Blair and Wolfe 2007). Although insect bodies and proboscises are known to transfer pollen in angiosperms, accounts of pollen transfer via wings are extremely rare (Cruden and Hermann-Parker 1979; Holmqvist et al. 2005). Furthermore, to the best of our knowledge, no report exists of wing-mediated pollen transfer in north temperate ecosystems.

The overarching goals of this study were to determine the role of butterfly wings in pollinating *R. calendulaceum* and to evaluate the extent to which the many different visitors to these flowers actually function as effective pollinators. To this end, we addressed the following questions: (1) Which insect visitors contact both male and female organs of *R. calendulaceum* flowers? (2) Do butterfly wings deposit flame azalea pollen on stigmas? (3) Are swallowtail butterflies the most important pollinators of flame azalea?

## Methods

This study was conducted during the summers of 2011 and 2014 at the University of Virginia's Mountain Lake Biological Station in southwestern Virginia (Giles County; 37°22′N, 80°32′W; elevation ∼1,160 m). All observations of flower visitors were performed on plants in an ∼0.5-km² area over 2 weeks in June 2011 and again in June 2014 during peak and postpeak flowering, as determined by qualitative observation of flowering phenology. Fruit set was evaluated August 4–6, 2014. Data for this study were deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad .th441 (Epps et al. 2015).

### Flower Visitor Behavior

We performed a series of field observations to evaluate the diversity of flower visitors to *Rhododendron calendulaceum* and to determine which visitors contact anthers and/or stigmas. We defined "flower visitor" as any nonherbivorous insect or hummingbird making contact with the upper side of open flowers and noted which visitors made contact with anthers and/or stigmas. In June 2011, we observed visitors to open flowers of eight flame azalea plants and recorded whether insects contacted anthers and/or stigmas. For butterfly visits, we documented the number of visits in which an insect contacted both the stigma and at least one anther. We also noted whether this contact with flower parts was made with the butterfly's body or wings. We performed additional observations of flower visitors on 26 flame azalea plants in June 2014, recording behaviors of visitors and noting any contact with anthers and/or stigmas. Observations were made over 10 days for a total of ∼17 h, primarily on sunny days (mean $T = 21$C). Unknown insects were collected or photographed if possible and identified to the finest level feasible, using keys or consultation with experts. We evaluated the relative efficacy of different butterfly species as pollinators, using Fisher's exact test to compare the probability of contact with anthers or stigmas.

### Butterfly Pollen Loads

To determine the relative pollen load carried on the wings versus bodies of butterflies, we captured eastern tiger swallowtail butterflies (*Papilio glaucus*) foraging on *R. calendulaceum* and placed them in glassine envelopes, after which they were frozen for study. We examined butterflies under a dissecting microscope and recorded the number of flame azalea pollen grains present on the body and lower surfaces of wings. Pollen grains of *R. calendulaceum* are connected by viscin threads and are easily distinguishable from pollen of coflowering species. Total individual pollen grains were counted on 17 butterflies, but for efficiency, pollen loads on wings and bodies of the remaining six butterflies (all

from 2014) were each assigned to one of nine categories, ranging between 0 and >7,500. We tested the hypothesis that butterflies carry more pollen on their wings than on their bodies with a paired Wilcoxon test, using the midpoint of each range for analysis. We also evaluated the relative density of pollen on butterfly wings versus bodies by photographing each butterfly from our 2014 collections and using ImageJ (Schneider et al. 2012) to measure the surface area of each insect's body (ventral and lateral surfaces) and wings (ventral surfaces).

### Stigmatic Pollen and Scale Loads

We examined naturally visited stigmas for the presence of butterfly wing scales and pollen to investigate an additional line of evidence for the role of butterfly wings in *R. calendulaceum* pollination. We collected 160 stigmas from open *R. calendulaceum* flowers (∼20 individual plants) over several days in June 2011. The stigmas were removed, attached to a microscope slide with adhesive tape, and examined under a dissecting microscope for the presence of pollen and/ or butterfly scales. In June 2014, we collected stigmas from an additional 18 naturally visited flowers immediately following a visit by a *P. glaucus* butterfly. Stigmas were collected from eight *R. calendulaceum* individuals, and stigmatic surfaces were examined for pollen and scales as described for 2011 collections.

### Stigma Loads from Single Insect Visits

To examine pollen and wing scale deposition onto *R. calendulaceum* stigmas following single insect visits, inflorescences of unopened flowers were cut from the field and kept in water in the lab. When flowers opened, we removed the anthers (carefully avoiding contact with the stigma) while leaving the filaments, stigma, and petals intact. These inflorescences were then attached to a pole and presented to *P. glaucus* butterflies foraging near flowering *R. calendulaceum* in the field. After a single insect visit, the stigma of the targeted flower was removed and secured to a microscope slide. We examined stigmas under a microscope within 24 h of collection and recorded the presence of pollen or wing scales.

### Pollinator Exclusion Study

In June 2014, we conducted a visitor exclusion experiment to determine the pollination efficiency of large flower visitors (which, based on our observations, were exclusively butterflies) versus other insect visitors to *R. calendulaceum* flowers. From June 12–14, we haphazardly assigned unopened inflorescences of 11 plants to the following treatments: (1) total visitor exclusion: inflorescences were enclosed in chicken wire covered with fine mesh screen ($N = 17$); (2) large visi-

tor exclusion (primarily butterflies; all nonbutterfly flower visitors observed were <1.5 cm): inflorescences were enclosed in a chicken wire cage with ~2.5-cm-diameter holes ($N = 17$); and (3) natural pollination: inflorescences were left open to allow unrestricted visitor access ($N = 14$). (4) In order to determine maximum fruit set, we conducted a series of hand-pollination crosses; budding inflorescences were covered loosely with a plastic bag until anthesis, when the bag was removed and flowers pollinated by hand using freshly collected outcrossed pollen ($N = 20$). Each of 10 plants received a full set of treatments, and an additional plant received all but the outcrossing treatment. We revisited all experimental plants August 4–6, 2014, and determined the proportion of successfully forming fruit in each inflorescence. Fruit production was considered successful if swollen capsules had formed subtending the calyx of flowers in targeted inflorescences. We used a Wilcoxon test to compare the effects of our treatments on the proportion of successful fruits formed in each inflorescence.

### Statistical Analysis

Analyses were performed in JMP 10 (Sall and Lehman 1996). Nonparametric tests were used when data were significantly departed from a normal distribution, as per the Shapiro-Wilk test.

### Results

#### Behavior of Flower Visitors

In 2011, we observed 40 swallowtail butterflies (*Papilio glaucus*) visiting 145 *Rhododendron calendulaceum* flowers. Butterfly wings were observed contacting the stigma and at least one anther in 57% of flower visits and with more than nine times greater frequency than contact between butterfly bodies and flower organs (two-tailed $P < .0001$ from Fisher's exact test; fig. 1*A*). A variety of solitary bees and a few bumblebees (*Bombus*) were observed visiting flowers but were not observed to contact more than one type of flower organ, even over successive visits (table A1, available online).

Our 2014 observations of flower visitors revealed 11 species and five orders of insects visiting *R. calendulaceum* flowers (table A1), though *Bombus* was not observed. Although most of these insects appeared to be foraging in the flowers, only five species were clearly observed to contact either the anthers or the stigmas. Of these, the most common were *P. glaucus* butterflies and *Andrena* bees (Halictidae). However, the large butterflies *P. glaucus* and *Speyeria cybele* were the only species ever observed to contact both stigmas and anthers (table A1). *Andrena cornelli* bees were active collectors of *R. calendulaceum* pollen, typically landing on filaments and climbing up to gather pol-
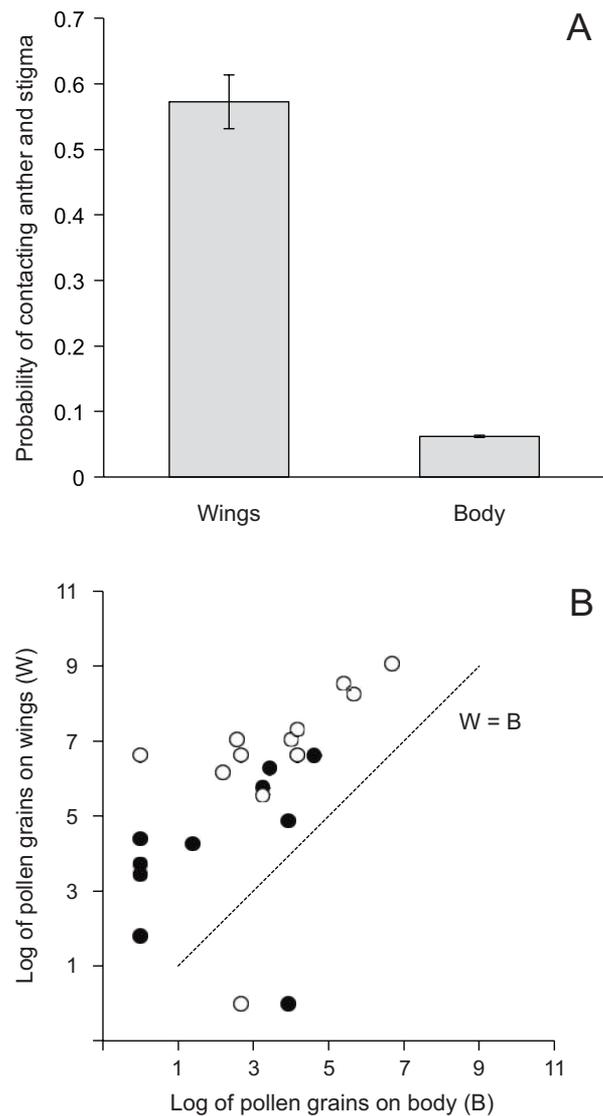


**Figure 1:** Evidence that butterfly wings are the primary vehicle for pollination in *Rhododendron calendulaceum* (flame azalea). *A*, Probability of a *Papilio glaucus* butterfly contacting both anthers and stigma with its wings versus its body during a visit to a flower. Probability of contact with both reproductive organs was significantly greater for butterfly wings than bodies ($P < .0001$, Fisher's exact test); error bars show standard error. *B*, Flame azalea pollen load carried on bodies versus wings of *P. glaucus* individuals. Filled and open circles represent butterflies sampled in 2011 ($N = 10$) and 2014 ($N = 13$), respectively. Points represent individual butterflies, and those positioned above the $W = B$ line indicate more pollen on a butterfly's wings than its body. Both axes show ln-transformed number of pollen grains $+ 1$. Data for this figure are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.th441 (Epps et al. 2015).

len from anthers. Because of the spatial separation between anthers and stigmas in *R. calendulaceum*, *A. cornelli* routinely removed large amounts of pollen without contacting

the stigma. At least two species of halictid bees were also common visitors but foraged on nectar or on the sticky stigmatic surface and did not contact the anthers (table A1).

We recorded butterfly contact with floral organs for 36 visits to flowers on five *R. calendulaceum* plants by *P. glaucus* (16 individuals) and 42 flower visits over a single plant by *S. cybele* (six individuals). Although we did not record the number of contacts made by butterfly bodies versus wings in these observations, almost all observed contacts with reproductive organs appeared to have been made with the wings. *Papilio glaucus* was twice as likely as *S. cybele* to contact an anther and seven times more likely to contact a stigma of *R. calendulaceum* during a flower visit (two-tailed $P < .0001$ from Fisher's exact tests; fig. 2).

### Butterfly Pollen Loads

We collected 10 (2011) and 13 (2014) foraging *P. glaucus* butterflies and quantified their pollen loads. Although *R. calendulaceum* pollen was found on all 23 butterflies sampled, the load was highly variable among individuals, ranging from five to more than 9,000 pollen grains. Butterflies bore 56.9 times more pollen on their wings than on their bodies, a pattern consistent across years ($W = 131.0$, one-tailed $P < .0001$ from paired Wilcoxon test for 2011 and 2014 combined; fig. 1*B*). On average ($\pm$ SD), captured swallowtails carried $82 \pm 171$ *R. calendulaceum* pollen grains on their bodies and $1{,}180 \pm 2{,}055$ on their wings. Pollen
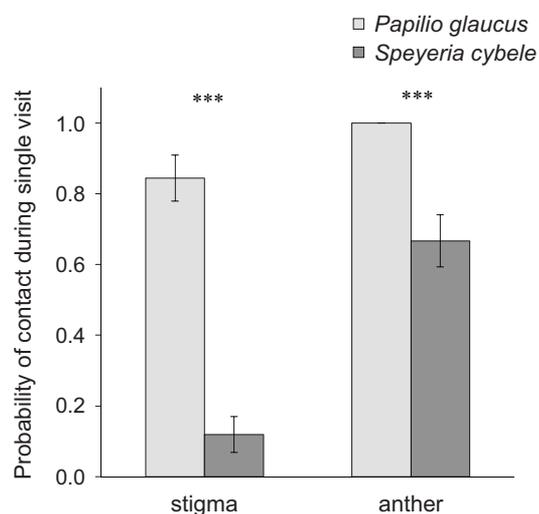


**Figure 2:** Probability of contact between the reproductive organs of *Rhododendron calendulaceum* and each of two butterfly species (*Papilio glaucus* and *Speyeria cybele*). Error bars show standard error; three asterisks indicate $P < .0001$. Data for this figure are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.th441 (Epps et al. 2015).

density was also 3.4 times greater on wings than on bodies ($W = 32.0$, one-tailed $P = .001$ from paired Wilcoxon test). The majority (70%) of butterflies collected had *R. calendulaceum* pollen on both their bodies and their wings.

### Stigmatic Pollen and Scale Loads

Butterfly wing scales and *R. calendulaceum* pollen were both found on flame azalea stigmas. Of the 160 stigmas examined in 2011, 10% had only pollen, 35% had only scales, and 10% had both pollen and wing scales present. In 2014, we collected 18 additional stigmas from flowers immediately following a visit by *P. glaucus* (nine individuals). Of these, 5% bore only pollen, 61% bore only scales, and 17% bore both pollen and wing scales.

### Stigma Loads from Single Insect Visits

Thirty-two stigmas (four in 2011; 28 in 2014) from virgin, emasculated flowers were examined after a single visit by *P. glaucus*. Of these, 47% had wing scales stuck to the stigmatic surface, 16% had pollen, and 9% had both.

### Pollinator Exclusion Study

The pollinator exclusion experiment showed strong variation in fruit set as a function of treatment (fig. 3). Fruit set was highest (mean $\pm$ SE: 57% $\pm$ 10% of flowers per inflorescence) in outcrossed inflorescences but did not differ significantly from that of inflorescences open to all flower visitors (27% $\pm$ 8% of each inflorescence; $Z = 1.63$, $P = .10$ from Wilcoxon test; fig. 3). No mesh-covered inflorescences (representing total exclusion of visitors) produced fruit, suggesting that *R. calendulaceum* may be incapable of self-pollination. We found no difference between mesh-covered inflorescences and caged inflorescences ($Z = 1.4$, $P = .16$ from Wilcoxon test; fig. 3), where caged inflorescences excluded large butterflies and potentially other large visitors; however, butterflies were the only large visitors we ever observed at *R. calendulaceum* flowers. However, two out of 78 caged flowers did produce fruit. Both caged and meshed inflorescences showed more than a 10-fold reduction in fruit set compared with open inflorescences ($Z = -3.08$, $P = .002$ and $Z = -3.76$, $P = .0002$ for caged and meshed treatments, respectively, from Wilcoxon tests; fig. 3). Fruit set was on average 12-fold higher in open inflorescences with butterfly access compared with caged inflorescences (fig. 3).

### Discussion

The floral display of flame azalea, *Rhododendron calendulaceum*, is visually striking and conspicuous in the forest
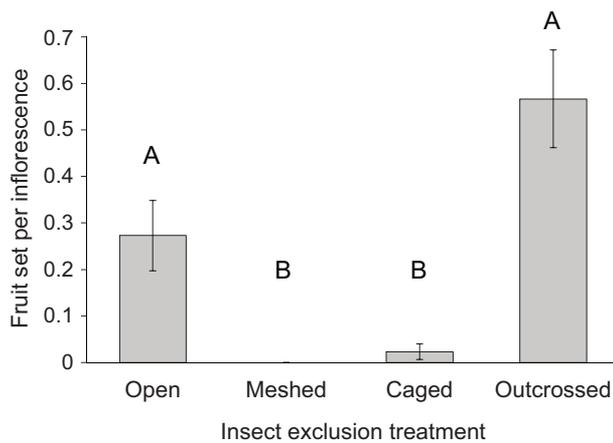
**Figure 3:** Fruit set in *Rhododendron calendulaceum* as a result of different pollinator exclusion treatments, showing the proportion of successful fruit formed out of the total flowers in each inflorescence as a function of treatment. Treatments are as follows: open: uncovered flowers with access to all visitors; meshed: inflorescence enclosed in fine mesh to exclude all visitors; caged: inflorescences caged to exclude only large visitors (i.e., butterflies); outcrossed: flowers hand-pollinated with outcrossed pollen. Error bars show standard error, and different letters indicate significant differences. Data for this figure are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.th441 (Epps et al. 2015).

understory, where the flowers attract a broad diversity of foraging insects. However, after ~10 years of observations (Wolfe and Rissler 1999; Blair and Wolfe 2007), it is clear that the vast majority of floral visits do not result in effective pollination. Indeed, fruit set has been found to be severely pollen limited in this species (Blair and Wolfe 2007). In addition, pollinator activity can be unpredictable in eastern forests in spring (Schemske et al. 1978), and the availability of flame azalea's pollinators varies widely between years (Blair and Wolfe 2007).

Pollen limitation in *R. calendulaceum* does not appear to result from a scarcity of potential pollinators, however, because virtually all flowers are visited (Blair and Wolfe 2007). Rather, the cause appears to be the ineffectiveness of flower visitors. Although several bee species commonly forage on the flower organs, these insects appear to specialize either on female (e.g., halictid bees) or on male (e.g., *Andrena*) flower parts without contacting both. Insects in the latter category, especially the pollen forager *Andrena cornelli*, may therefore remove a substantial amount of pollen from the flame azalea pollen pool without contributing to pollination. Out of the diverse assemblage of insects visiting *R. calendulaceum* flowers, only two large butterflies (*Papilio glaucus* and *Speyeria cybele*) were observed to contact both male and female reproductive organs. Although these insects usually enter the flower at an angle such that

their bodies miss the exserted anthers and stigma, their flapping wings make solid contact with both.

Experimental exclusion of flower visitors clearly showed that apart from butterflies, most visitor species are indeed ineffective as pollinators. No fruit was produced by mesh-covered flowers, suggesting that *R. calendulaceum* is incapable of selfing. However, only 2% of flowers in the caged treatment group successfully formed fruit, in contrast to nearly one-third of flowers with access to butterflies (fig. 3). This suggests that successful pollination is nearly eliminated in the absence of butterflies. Although the presence of a wire cage could have deterred some insects, we observed visitors such as *A. cornelli* foraging readily within the exclosures, suggesting that deterrent effects on small visitors were minimal. Of the many visitors observed on flame azalea flowers, *P. glaucus* and *S. cybele* were the only species large enough to be impeded by the wire cage. Although hummingbirds also would be excluded, in all our work we noted only a single brief inspection of a flame azalea flower by a ruby-throated hummingbird, which involved no actual contact with the plant. For this reason, we believe that this species is unlikely to contribute significantly to *R. calendulaceum* pollination at our study site.

Complementary to these findings, other results point to large butterflies as frequent and effective pollinators of flame azalea. *Rhododendron calendulaceum* pollen was present on all *P. glaucus* butterflies sampled, with the average individual carrying well more than 1,000 pollen grains. Stigmas examined from *R. calendulaceum* flowers with unknown visitor history showed that nearly half bore wing scales, a clear sign of having been contacted by a butterfly wing. Finally, the repeated transfer of pollen to the stigmas of virgin flowers during visits from *P. glaucus* demonstrated that these insects readily perform pollination. Furthermore, of the two butterfly species observed visiting azalea flowers at our study site, *P. glaucus* may contribute substantially more to pollination than *S. cybele*. Although we observed both species make contact with stigmas and anthers, interaction with either organ was far more likely during visits by *P. glaucus* than by *S. cybele* (fig. 2). This discrepancy in pollinator effectiveness (as measured by probable contribution to pollen deposition; Ne'eman et al. 2010) appears to derive from differences in foraging behavior. While *P. glaucus* tends to flap its wings continuously, brushing them repeatedly onto flower organs, *S. cybele* flaps little and most often contacts reproductive parts only when arriving at or leaving a flower (video 1, available online). In addition to being a more effective pollinator, *P. glaucus* was far more abundant on *R. calendulaceum* than *S. cybele*, which was observed only occasionally. Indeed, previous work involving 2 years of visitor observations on flame azalea flowers at our study site reported no sightings of *S. cybele* (Blair and Wolfe 2007). Hence, we conclude that as pollinators of flame azalea,

**Video 1:** Still photograph from a video (video 1, available online) that shows a great spangled fritillary (*Speyeria cybele*) foraging on *Rhododendron calendulaceum*. The video clip illustrates the differences in wing-flapping behavior between *S. cybele* and eastern tiger swallowtails (*Papilio glaucus*) while foraging for nectar on *R. calendulaceum*. *Papilio glaucus* butterflies flap their wings continuously while feeding, making frequent contact with anthers and stigmas. In contrast, *S. cybele* typically flaps its wings only when taking flight, making this species a relatively inefficient pollinator of *R. calendulaceum*.

*P. glaucus* scores far above *S. cybele* in the metric of pollinator importance (as defined by the product of visitation frequency and pollinator effectiveness; Ne'eman et al. 2010).

Together, our findings demonstrate that large butterflies—and especially *P. glaucus*—perform nearly all pollination services in *R. calendulaceum*. This specialization on butterfly pollinators exists despite the presence of a wide range of flower visitors, many of which regularly contact one of the flower's reproductive organs. Although pollinator efficiency is known to vary among animal species (Schemske and Horvitz 1984; Conner et al. 1995; Ollerton et al. 2007), our study demonstrates almost complete failure of female flame azalea reproductive success in the absence of large butterflies.

The pollination literature contains abundant examples of highly generalized pollination networks based on flower visitor data (e.g., Petanidou et al. 2008; Junker et al. 2010), and such studies have contributed much to our understanding of properties such as interaction strengths and temporal variation in community-level interactions. However, our results contribute to a growing body of work that suggests a need for caution in classifying flower visitors as pollinators, at least when the goal is to reconstruct truly mutualistic pollination interactions (Alarcón 2010; King et al. 2013). The fact that visitation does not necessarily equate with successful pollination has been widely discussed (Fumero-Cabán and Meléndez-Ackerman 2007; Popic et al. 2013), leading to attempts by many authors to minimize the discrepancy between visitor networks and true pollination networks. Of these, the most common strategy is to score only those

flower visitors seen to contact an anther or stigma (Memmott 1999; Burkle and Irwin 2009; Stang et al. 2009). However, in our study, several visitors routinely contacted just the anthers or stigmas without contacting the opposite organ, as required for pollination. Recent attempts to quantify pollen transport networks have been more rigorous (Popic et al. 2013; Alarcón 2010), but our results (albeit limited in scope to observations of a single population of one plant species) suggest that even these do not necessarily identify mutualistic interactions with pollinators. For example, although *A. cornelli* is a dominant visitor to flame azalea flowers and carries large amounts of their pollen, this bee appears to be a pollen thief and rarely (if ever) contacts the stigma. The formation of two fruits in our large visitor exclusion treatment suggests that smaller pollen-carrying visitors such as *A. cornelli* may pollinate in rare cases. However, our data indicate that this species contributes far more to pollen limitation than to pollination; thus, its interactions with azalea are primarily antagonistic.

The most reliable way to distinguish actual pollinators from a flower visitor community may be to quantify the pollen deposited on virgin stigmas after a visit (King et al. 2013); however, this is often impracticable in community-level studies. Consequently, an effective alternative may be to score species as pollinators only after observing repeated contact with both male and female reproductive parts. Such observations would be especially effective if performed in concert with pollen transport data. Admittedly, this level of observation may be a challenging task for some communities, and contact may be difficult to spot for rare visitors or those with unusually short flower visits. Where feasible, however, this method may provide the most accurate estimation of mutualistic pollination networks for whole communities.

### The Role of Butterfly Wings in Azalea Pollination

Although a large fraction of angiosperm species are pollinated by flying animals, wings themselves are not typically viewed as pollen vectors. Our results strongly suggest that butterfly wings are the principal vehicle of pollination in *R. calendulaceum*. This conclusion is supported by our findings that butterflies were much more likely to contact both stigmas and anthers with their wings than with their bodies and that greater than 50-fold more flame azalea pollen was carried on butterfly wings than on bodies (fig. 1*B*). In addition, a substantial proportion of *R. calendulaceum* stigmas carried both wing scales and pollen.

Butterfly wings offer a large surface area for contact with the exserted reproductive parts of *R. calendulaceum*, and the flapping of wings during foraging enables frequent contact with both anthers and stigmas. In addition to transferring pollen, however, we found that butterflies may de-

posit wing scales on stigmas with even greater frequency. Although not directly examined in our study, it is possible that this ready transfer of wing scales could come at some cost to pollination efficiency (e.g., by clogging the stigmatic surface). Our observations also suggest that differences in wing-flapping behavior may affect the relative efficiency of different butterflies as pollinators, as described above for *P. glaucus* and *S. cybele*. Although the haphazard flapping of a wing may appear more random and less efficient than the body as a vehicle for pollen transport, the viscin threads connecting flame azalea pollen grains may increase the efficiency of the wing as a pollen vector. When a wing contacts the poricidal anther of *R. calendulaceum*, long threads of multiple pollen grains are pulled out, a process we mimicked in the laboratory by pressing the wing of a swallowtail onto anthers. This removal pattern resulted in a clumped distribution of large numbers of pollen grains on the wing. Consequently, even if the probability of contact between the stigma and a single pollen grain is low, the transfer of clumped pollen may increase the chances for fertilization. This strategy in *R. calendulaceum* is remarkably similar to that of the small tree *Caesalpinia pulcherrima* (Fabaceae) of tropical Mexico. This plant also produces large showy flowers whose pollen is similarly held together by viscin threads, and it is pollinated by butterflies that carry clumped pollen on their wings (Cruden and Hermann-Parker 1979). A second case of pollen transfer on butterfly wings was recently described in *Clivia miniata* (Amaryllidaceae), a herbaceous perennial in the South African highlands (Kiepel and Johnson 2014). This plant also produces large clusters of reddish orange flowers similar to those of *R. calendulaceum* and *C. pulcherrima*. To our knowledge, these are the only prior reports of wings as pollen vectors.

Although many insects contact the anthers or stigmas of *R. calendulaceum*, this species appears to be remarkably specialized on pollination by the flapping wings of butterflies and primarily those of a single species. In addition to demonstrating a case of narrow specialization in a plant with diverse insects contacting its reproductive organs, this study offers implications for a potentially overlooked mode of pollen transfer on the wings of flower visitors. Butterflies are often considered poor pollinators (Wiklund et al. 1979), but their wings offer a ready surface with which to contact the long-exserted anthers and stigmas of *R. calendulaceum*. Although this study presents only the third report of wing-mediated pollination, the transfer of pollen by butterfly wings may not be a rare event. Many plant species have long-exserted reproductive parts, which may lend themselves well to the removal and deposition of pollen by flapping butterfly wings, much as we see in flame azalea. Thus, future studies might do well to examine butterfly wings as a possible pollen vector in other plant species.

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*Left*, an azalea bee (*Andrena cornelli*) foraging on flame azalea flowers. This bee removes large amounts of pollen from flame azalea but was not observed to contact the female flower part (the stigma) as is necessary for pollination. Photo credit: Suzanne E. Allison. *Right*, flowers of the flame azalea (*Rhododendron calendulaceum*). Photo credit: Mary Jane Epps.