VOL. 191, NO. 6 THE AMERICAN NATURALIST JUNE 2018

NATURAL HISTORY MISCELLANY NOTE

Dispensing Pollen Via Catapult: Explosive Pollen Release in Mountain Laurel (*Kalmia latifolia*)

Callin M. Switzer,[1,*] **Stacey A. Combes,**[2] **and Robin Hopkins**[1]

1. Department of Organismic and Evolutionary Biology, Arnold Arboretum, Harvard University, Boston, Massachusetts 02130;
2. Department of Neurobiology, Physiology and Behavior, University of California, Davis, California 95616

Submitted July 18, 2017; Accepted November 15, 2017; Electronically published March 22, 2018

Online enhancements: appendixes, videos, zip file. *Dryad data:* http://dx.doi.org/10.5061/dryad.65sd4.

ABSTRACT: The astonishing amount of floral diversity has inspired countless assumptions about the function of diverse forms and their adaptive significance, yet many of these hypothesized functions are untested. We investigated an often-repeated adaptive hypothesis about how an extreme floral form functions. In this study, we conducted four investigations to understand the adaptive function of explosive pollination in *Kalmia latifolia*, the mountain laurel. We first performed a kinematic analysis of anther movement. Second, we constructed a heat map of pollen trajectories in three-dimensional space. Third, we conducted field observations of pollinators and their behaviors while visiting *K. latifolia*. Finally, we conducted a pollination experiment to investigate the importance of pollinators for fertilization success. Our results suggest that insect visitation dramatically improves fertilization success and that bees are the primary pollinators that trigger explosive pollen release.

Keywords: explosive pollination, bumblebee, biomechanics, pollen dispersal.

Introduction

The sheer magnitude of floral diversity has puzzled and intrigued botanists for hundreds of years (Darwin 1862*b*; Galen 1999; Friedman 2009). Sometimes explanations for how and why flower forms function, while untested, are repeated, leading to a mythology of adaptive explanations (Gould and Lewontin 1979). These adaptive hypotheses are often only partially correct for several reasons. First, initial observations may be misleading—without fully characterizing how diverse flower forms function, we have an incomplete picture of the interaction between pollinators and plants. Second, floral traits are under many different selective pressures (Galen 1999) and thus are unlikely to be optimized for a single function (Gould and Lewontin 1979), such as pollinator attraction. Scientists have thoroughly investigated a few of these adaptive explanations (Miller 1981; Schemske and Horvitz 1984; Nilsson 1988; Hurlbert et al. 1996), and several of these have turned out to be incorrect (e.g., Wilson 1995; Temeles and Rankin 2000). These misinterpretations of floral form suggest that further investigations of function are necessary to understand the adaptive significance of flower characteristics (Poppinga et al. 2010).

Here we thoroughly investigate the explosive pollination mechanism in *Kalmia latifolia*, mountain laurel, as a case study of one of the most charismatic examples of extreme floral form with multiple hypothesized functions. Our work has important implications for understanding the adaptive significance of this flower trait and represents an integrative approach to understanding the function of diverse forms.

Explosive Pollination

Explosive pollination mechanisms have evolved in several plant families, including Lamiaceae, Fabaceae, Rhizophoraceae, Onagraceae (Raju and Reddi 1995; Solomon and Rao 2006), and Marantaceae (Pischtschan and Claßen-Bockhoff 2008), and thus the function of this trait may differ for each lineage. For instance, some wind-pollinated plants, such as the white mulberry tree (Taylor et al. 2006), are thought to use explosive pollination to launch pollen into the air—thereby allowing the pollen to travel great distances. In other plants, flowers can actively respond to the presence of a pollinator and forcibly attach pollen to the unsuspecting visitor, which Darwin (1862*b*) originally termed "sensitiveness." Darwin (1862*b*), for example, was fascinated by how *Catasetum* orchids launched pollinia onto bee visitors. Several plants in Papilionoideae (*Desmodium* spp.) also catapult their pollen in response to bees (Alemán et al. 2014; Fleming and Etcheverry 2017). *Catasetum* and *Desmodium* are examples of how explosive pollination may increase pollinator-mediated pol-

---

* Corresponding author. Present address: Department of Biology, University of Washington, Seattle, Washington 98195; e-mail: callin.switzer@gmail.com.

ORCIDs: Switzer, http://orcid.org/0000-0003-0901-169X; Combes, http://orcid.org/0000-0003-1586-6459; Hopkins, http://orcid.org/0000-0002-6283-4145.

Am. Nat. 2018. Vol. 191, pp. 000–000. © 2018 by The University of Chicago. 0003-0147/2018/19106-57830$15.00. All rights reserved.
DOI: 10.1086/697220

len transfer efficiency. For some plants, the function of explosive pollination has evolved for multiple pollination mechanisms. For example, flowers of the bunchberry dogwood, *Cornus canadensis*, can be triggered by pollinator visits, or they can catapult pollen autonomously (Edwards et al. 2005).

Researchers have quantified aspects of the biomechanics of explosive pollination in some of these plants. For instance, the maximum speed for flying pollinia from *Catasetum fimbriatum* has been reported as averaging 2.2 m s^{-1} (Fulop 2009), 2.65 m s^{-1} (Nicholson et al. 2008), and 2.76 m s^{-1} (Ebel et al. 1974). Flowers of *C. canadensis* can launch dry grains of pollen with a maximum speed of 3.1 m s^{-1} and accelerate pollen at 24,000 m s^{-2} (Edwards et al. 2005). Explosive pollination has been discussed in the literature for more than 150 years (Darwin 1862*b*; Beal 1867; Rothrock 1867), and yet there is still little known about what triggers pollen release how plants eject, catapult, or explode their pollen.

Adaptive Significance

Like many plants with this floral trait, the adaptive significance of explosive pollination is not fully understood for *K. latifolia*. Inspired in part by Darwin's (1862*b*) work on pollination, two American botanists, J. T. Rothrock (1867) and W. J. Beal (1867), described the pollen movement mechanism of *K. latifolia* and hypothesized different functions. Rothrock (1867) suggested the pollen was aimed at the stigma in a highly elaborate effort to ensure self-pollination. Beal (1867), on the other hand, observed floral visits by bees and suggested that the pollen was catapulted onto the bee for cross-fertilization purposes. There is still no consensus as to which adaptive explanation is true in this system. Some subsequent research reports bumblebees as common pollinators (Jaynes 1988; Rathcke 1988; Real and Rathcke 1991; Rathcke and Real 1993; Nagy et al. 1999; Mathews and Collins 2014) and generally argues for the role of insects in successful pollination (Nagy et al. 1999), while other research suggests insect pollinators are not necessary as *K. latifolia* flowers may catapult pollen without being triggered by insect visitors (Rathcke and Real 1993). Here we provide a multifaceted evaluation of the explosive pollination mechanism in *K. latifolia*. Our study addresses four specific research questions: (1) How fast (speed and acceleration) is the pollen catapult? (2) Where does the catapulted pollen travel? (3) What pollinators and behaviors trigger the catapult? (4) What role do pollinators play in fertilization? To the best of our knowledge, this research provides the most comprehensive understanding of how explosive pollination works and why it might be effective in *K. latifolia*. This work exemplifies how investigating the function of an elaborate floral form can provide insights into its adaptive significance.

Methods

Study Organism

Kalmia latifolia is a perennial shrub that is native to the eastern United States, ranging from Maine to Florida (USDA NRCS 2017). Flowers of *K. latifolia* have fused petals with 10 radially aligned stamens. Each anther is inserted into a pocket in the corolla and held under tension by a curved filament until it is triggered (Jaynes 1988; Rathcke and Real 1993; Nimmo et al. 2014; fig. 1*A*, 1*B*). As the flower matures, the petals flex backward, and the filaments are bent "much like a clock's mainspring" (Niklas 1992, p. 109). This generates strain energy that is stored in the filaments as potential energy (Niklas 1992). When an anther is released from a pocket, the filament rapidly straightens, and pollen is launched into the air (video A1; videos A1–A8 are available online). Each anther dehisces by two apical clefts, which are open before the flower fully opens (Hermann and Palser 2000). These clefts point roughly in line with the radial axis (on the distal portion of the anther sac). The structure of the anther sac and position of the anther openings probably play a key role in pollen release. The anthers can be triggered on the same day that the flower opens (C. M. Switzer, personal observation). Pollen grains form tetrads that are connected with viscin threads (Hermann and Palser 2000; Sarwar and Takahashi 2012), causing each anther to release several stringy aggregations of pollen when it is triggered.

Study Location

All observations and experiments were conducted with *K. latifolia* plants located in the Arnold Arboretum. The Arnold Arboretum is 281 acres of land that is about 7.5 km from the highly developed downtown area of Boston, Massachusetts (for accession numbers and more information, see "Arboretum Information"; fig. C1; figs. A1, A2, B1–B5, and C1 are available online). The habitat in which *K. latifolia* grows in the Arnold Arboretum is similar to the habitat found in nearby forests of the northeastern United States; in fact, many wild *K. latifolia* plants can be found growing within the Arnold Arboretum. The living collections in the Arnold Arboretum consist of more than 14,000 accessioned plants, largely woody species of North America and eastern Asia. We chose to conduct research in the Arnold Arboretum because it is an ideal location to study plants in an outdoor setting, while still providing access to a lab space that could be used for conducting controlled experiments, recording high-speed videos, and recharging the camera batteries. Of note, the population of *K. latifolia* in our study is not representative of the geographical location; the Arnold Arboretum contains a variety of accessioned plants from different locations. The Arnold Arboretum likely has coflowering plants different from those in the surrounding communities—this

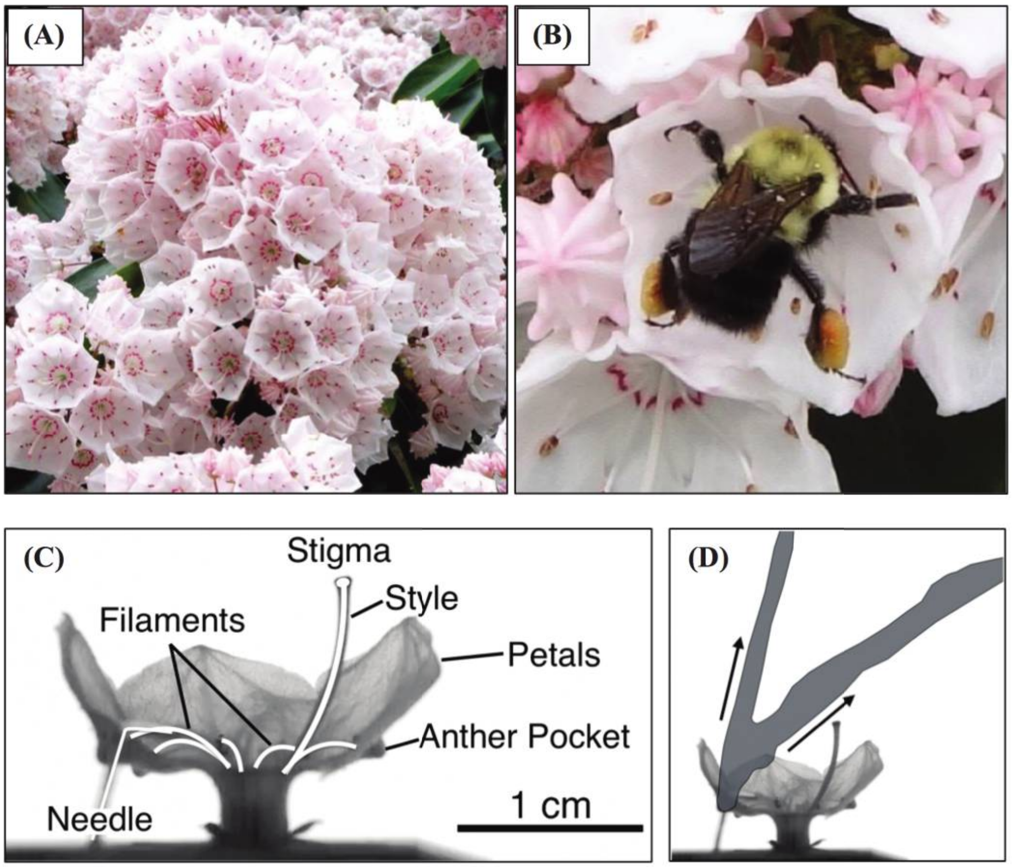

Figure 1: *Kalmia latifolia* flowers and pollen trajectory. Including photos of inflorescences (*A*) and a bumblebee visiting a flower (*B*). *A*, *B*, Photos by William (Ned) Friedman. *C*, Side view of *K. latifolia* flower before releasing pollen. The stigma, style, filaments, and the needle used for manually triggering the catapult have been traced in white. The stigma is off-center, and half of the petals have been removed to allow for visualization of the pollen release. *D*, Flower with gray polygon showing the area pollen passed through after being catapulted from the anther. The arrows indicate the direction that pollen flew from the catapult.

could influence the pollinator community, since coflowering shrubs can cause competition for pollinators (Rathcke 1988; Rathcke and Real 1993).

Pollen Speed and Acceleration

Our first goal was to quantify the maximum speed and acceleration of pollen. We characterized the kinematics of the *K. latifolia* pollen catapult by manually triggering filaments with a needle in the lab (video A1). We recorded pollen release at 5,000 frames s^{-1} with a high-speed video camera (FASTCAM SA3 with a 105 mm f/2.8 lens; Photron, San Diego, CA) positioned orthogonal to the plane of pollen release (see examples in video A1). We removed a section of petals to visualize the pollen trajectory. We recorded 32 pollen-catapult events from seven flowering individuals collected in June 2015.

Each anther released multiple aggregations of pollen, but we focused this analysis on only the fastest pollen clump or part of a clump. We calculated the position of pollen at every time step by digitizing the videos using the MATLAB-based program DLTdv5 (Hedrick 2008; MATLAB R2014b, Natick, MA). We smoothed the position measurements to reduce digitization errors (see app. B; apps. A and B are available online).

Calculating Pollen Trajectories in 3-D Space

Our second goal was to determine where the pollen travels after release and thus determine where a pollinator should be located in order to be hit by pollen. Using the method described above, we triggered pollen release from 29 flowers from 20 individuals in June 2016 (see video A1). For these analyses, we visualized the entire region that pollen traveled within 2–3 cm of the flower (fig. 1*C*, 1*D*). We digitized a polygon around the area of released pollen with custom-written code in MATLAB (see app. A for details). Figure 1*D* shows an example of the region that pollen occupied during a trial (also see video A1). We scaled and aligned all of the digitized polygons using custom-written scripts in R (R Core Team 2016) and Python (Python Software Foundation 2016; see fig. A2 and "Additional Methods for 3-D Heat Map").

We extruded the digitized pollen polygons to a thickness of 2 voxels (approximately the width of the anther), allowing us to visualize them into 3-D space (200^3 voxels; video A2). Finally, we performed a resampling bootstrap analysis to generate a heat map of pollen location in 3-D space. We resampled the 3-D extruded polygons (treating each as an independent observation), with replacement, and rotated each one in a randomly assigned increment of 1/10 of a full rotation (multiples of 36°). Each *K. latifolia* flower has 10 anthers, but we triggered only one per flower; thus, the random rotation allowed for a 3-D representation of pollen trajectory from the entire flower. We conducted this resampling routine 500 times. We averaged the resampled positions to produce a 3-D heat map of pollen trajectories. The heat map and resampling was done with custom-written scripts in Python (Python Software Foundation 2016) with the module Mayavi (Ramachandran and Varoquaux 2011).

Monitoring Insect Visitors and Causes of Catapulting Pollen

Our third goal was to document insects' visitation and behavior on *K. latifolia*. We collected high-speed videos (2,000 frames s^{-1}; Fastec TS4 with a 105 mm f/2.8 lens; Fastec Imaging, San Diego, CA) of insects visiting *K. latifolia* at the Arnold Arboretum between 08:00 and 12:00 on June 15, 16, 22, 23, and 30 in 2016. Our video recording procedure was aimed at collecting the largest number of videos, given the limited battery life of our high-speed camera (~4 h). We chose to film during the morning on sunny days because these conditions provided the bright light needed for recording. Furthermore, insect visitors were more prevalent in the mornings. Because there were relatively few insect visitors, we chose to manually search for insects that were visiting *K. latifolia* flowers and hold the camera for filming. After being filmed, we captured the pollinators and marked them with paint (oil-based paint pens; Sharpie, Oak Brook, IL) so that we could exclude pollinators that we had already filmed. We identified the pollinator species when possible, using the collected videos. Though long-term video techniques (e.g., Steen et al. 2011) would likely provide more accurate information about which pollinators visited the *K. latifolia* flowers, that approach was not feasible for high-speed videography. For each high-speed video, we identified the insect visitors and observed when visitors triggered anthers. We recorded a total of 69 insect visits (see videos A3–A5 for examples).

Pollination Experiments

Our final goal was to determine whether pollinators improve sexual reproductive success in *K. latifolia* in the Arnold Arboretum. Similar experiments have been conducted in the past with populations of *K. latifolia* in Virginia and Rhode Island (Rathcke and Real 1993; Nagy et al. 1999). However, the extent to which pollinators affect reproductive success may be different for different populations (Rathcke and Real 1993). On each of 22 plants, we first selected four inflorescences that were at approximately the same level of maturity and equally sized. After selecting inflorescences, we randomly assigned each to one of four treatments: (1) Control flowers were left open to all pollinators and not manipulated. (2) Flowers were bagged with tulle (wedding veil) to exclude all pollinators larger than ~1 mm in width. Hereafter, this group is referred to as autogamous selfed. (3) Flowers had self-pollen applied to receptive stigmas using forceps (forceps were sterilized with isopropyl alcohol between flowers) and were bagged with tulle to prevent pollinator access. This group will be referred to as manipulated selfed. (4) Flowers were left open to all pollinators and had additional, non-self-pollen added to receptive stigmas using forceps. This group will be referred to as supplemental outcrossed. Supplemental pollen was collected haphazardly from 10–20 other individuals, mixed together, and then placed on the stigmas of flowers. We continuously monitored the experimental plants (every 3–4 days) and applied treatments to all new flowers that opened since our last application of treatments. We applied treatments between 08:00 and 10:00 on June 9, 13, 16, 20, 23, 27, and 30 and July 4 and 7, 2016.

On October 20, 2016, we collected, cleaned, and photographed 1,305 fruits from our treatments. We used custom-written software and the module OpenCV (Bradski 2000) to automatically count fruits from images and measure diameters (see "Additional Notes on Code"). We used linear mixed models (LMMs) to compare fruit size and generalized linear mixed models (GLMMs) to compare the number of fruits in each of the treatments. For all mixed models, we used the library, lme4 (Bates et al. 2015). We included plant ID (accession number, $n = 22$) and plant lineage ($n = 17$, within the 22 plant IDs) as random effects in these models. Plants from the same lineage are related and are thus expected to have correlated traits (for accession numbers, see "Arboretum Information"); we included plant lineage to attempt to account for this relatedness. We used a negative binomial model to compare the counts of fruits in each treatment and a Gaussian model to compare the size of fruits. After constructing models, we calculated 95% bootstrap confidence intervals for the means (using 1,000 replications). The confidence intervals were based on fixed effects only.

We used the size of fruits as a proxy for the number of seeds produced. We confirmed that larger fruits produce more seeds by counting the number of seeds in one of the five carpels in one fruit from each of 19 individual plants that were used in the study (fruits were from flowers that were untreated). We used a Poisson GLMM with plant lineage as a random effect to confirm that fruit size was a significant predictor of the number of seeds.

Results

Pollen Speed and Acceleration

We found that the average maximum speed for *Kalmia latifolia* pollen was 3.5 m s^{-1} (95% confidence interval [CI]: 3.1–4.0), and the average maximum acceleration of pollen was 4,100 m s^{-2} (95% CI: 3,300–5,300). Data used for this and all further analyses are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.65sd4 (Switzer et al. 2018).

Pollen Location in 3-D Space

Pollen usually flew through the air in several stringy aggregations. In some trials, part of a pollen string stayed attached to the anther (video A6). Figure 2 shows 3-D contours and a 2-D contour map of a single slice through the 3-D heat map (for the 3-D heat map, see videos A7 and A8). The choppiness of the 3-D contours in figure 2 is due to the low resolution of the 3-D space (200^3 voxels). We

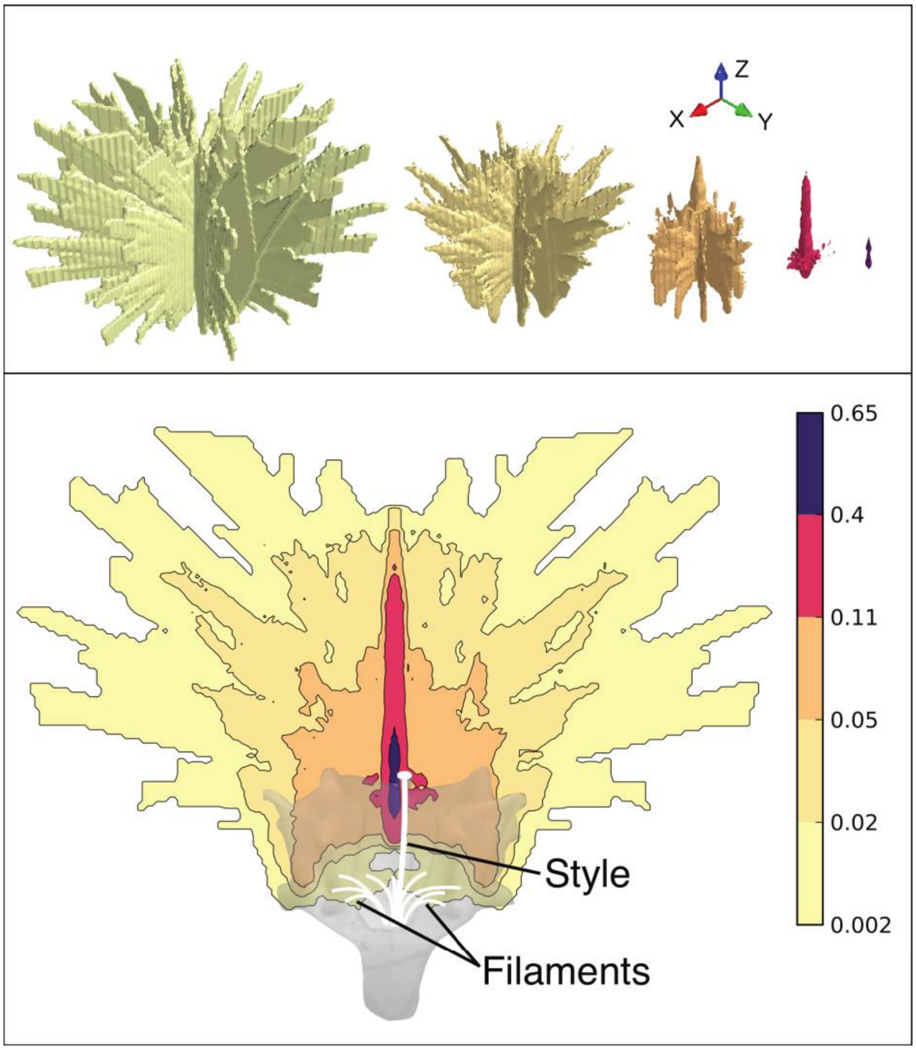

Figure 2: *Top*, 3-D visualizations of contours of pollen trajectories of *Kalmia latifolia*. *Bottom*, 2-D contour map (a slice through the 3-D map) of resampled pollen trajectories showing that pollen generally launches toward the center of the flower. The contours represent probability that pollen will fly through that region. For example, the darkest area in the center of the bottom figure indicates that pollen crossed through this area in 40%–65% of the trials. The overlaid flower is a guide to show the approximate position of the flower, and the filaments and style have been roughly traced with white lines.

found that catapulted pollen was most likely to cross the central axis of the flower at a height slightly greater than the length of a style. A single-anther heat map and images of all pollen trajectories can be found in figures B4 and B5.

Insect Visitors and Causes of Catapulting Pollen

We reviewed each of the 69 videos and report a brief summary of the pollinators (table 1). We found a variety of bee, butterfly, and wasp visitors, but the most common visitors were bumblebees, or *Bombus* spp. (54 of 69). We observed *Bombus impatiens* and several other species of bumblebees. We were also able to identify carpenter bees (*Xylocopa virginica*) and honeybees (*Apis mellifera*). We observed that only bees (bumblebees, honeybees, and carpenter bees) triggered the anthers to release pollen. With high-speed videos, we counted the number of triggered anthers, though sometimes we weren't able to see all anthers, due to the insect's body or the flower blocking the view. We observed insects triggering between zero and five anthers per visit. We documented bumblebees releasing the catapult ~40% of the time. They triggered the anthers by pulling the filaments toward their bodies with their front or mid legs as they landed on the flower or as they tried to push their proboscis deeper into the floral nectary. In every video, we observed bees extending their proboscises and pushing their heads into the center of the flowers—this suggests that bees were visiting *K. latifolia* mostly for nectar and not pollen. We saw bees grooming the pollen on their bodies, but we did not observe any bees attempting to collect only pollen. Large bees (bumblebees, carpenter bees, and occasionally honeybees) also typically contacted the stigma of the flower while they were searching for nectar. Figure 1*B* shows a bumblebee visiting a *K. latifolia* flower, where the stigma is underneath the bee in the photo. Videos A4 and A5 show examples of bumblebees visiting the flowers. We did not observe any bees smaller than honeybees visiting *K. latifolia*. Other insects may contact the stigma, for instance, moths and butterflies, but their wings obscured our view of the stigma. Wasps (potter wasps; subfamily: Eumeninae; see video A3) were observed collecting insect larvae from around the *K. latifolia* flowers, but we did not observe them feeding on nectar ($n = 3$). Butterflies could easily insert their long, slender proboscis into the corolla from a distance without triggering the anthers ($n = 4$). More detailed data is available in a zip file online.

Table 1: Counts of insect visitors filmed on *Kalmia latifolia* show that the majority of visitors were bumblebees and that only bees were observed triggering the catapult to release pollen

Insect	Visits	Visits that triggered catapult
Bumblebees (*Bombus* spp.)	54	22
Honeybees (*Apis mellifera*)	7	2
Butterflies and moths (order: Lepidoptera)	4	0
Potter wasps (subfamily: Eumeninae)	3	0
Carpenter bee (*Xylocopa virginica*)	1	1
Total	69	25

Pollination Experiments

Our final goal was to determine whether pollinators improved *K. latifolia*'s reproductive success. We found that the treatment (control, autogamous selfed, manipulated selfed, and supplemental outcrossed) significantly affected the number of fruits, while accounting for variation that was due to plant ID and lineage (negative binomial GLMM, $\chi^2_3 = 52.73$, $P < .001$). Figure 3*A* shows that autogamous-selfed inflorescences had the lowest fruit count, control and manipulated-selfed inflorescences had higher fruit counts, and supplemental-outcrossed inflorescences had the highest fruit count. Treatment also significantly affected the size of fruits that were produced, while accounting for the variation that came from plant ID and lineage ($\chi^2_3 = 389.3$, $P < .001$; fig. 3*B*). We found the same general relationship between the treatments and fruit size as above. When comparing treatments, we found that inflorescences that were excluded from pollinators (autogamous-selfed treatment) had smaller and fewer fruits than any of the other treatments (fig. 3*A*). Supplemental-outcrossed flowers showed increased fruit number and size, relative to other treatments (fig. 3). Manipulated-selfed flowers produced fruit numbers and sizes similar to those of the control group (fig. 3). We found that the size of the fruit (diameter) was a significant predictor of the number of seeds per carpel (Poisson GLMM, $\chi^2_1 = 21.0$, $P < .001$). Larger fruits contained a greater number of seeds.

Discussion

We set out to better understand the function and adaptive significance of an extreme floral form. The adaptive significance of many floral structures has been hypothesized and yet many of these hypotheses remain untested. Indeed, some of these assumed adaptive functions have been tested and found to be inaccurate generalizations (Wilson 1995; Temeles and Rankin 2000), which motivates further research in this area. In this study, we investigated the function of explosive pollination in *Kalmia latifolia*. Initial observations in this system resulted in misleading hypotheses about how pollen is catapulted into the air in order to land on a receptive stigma, rather than being transferred by the insect (Rothrock 1867). Indeed, in many of our personal observa-

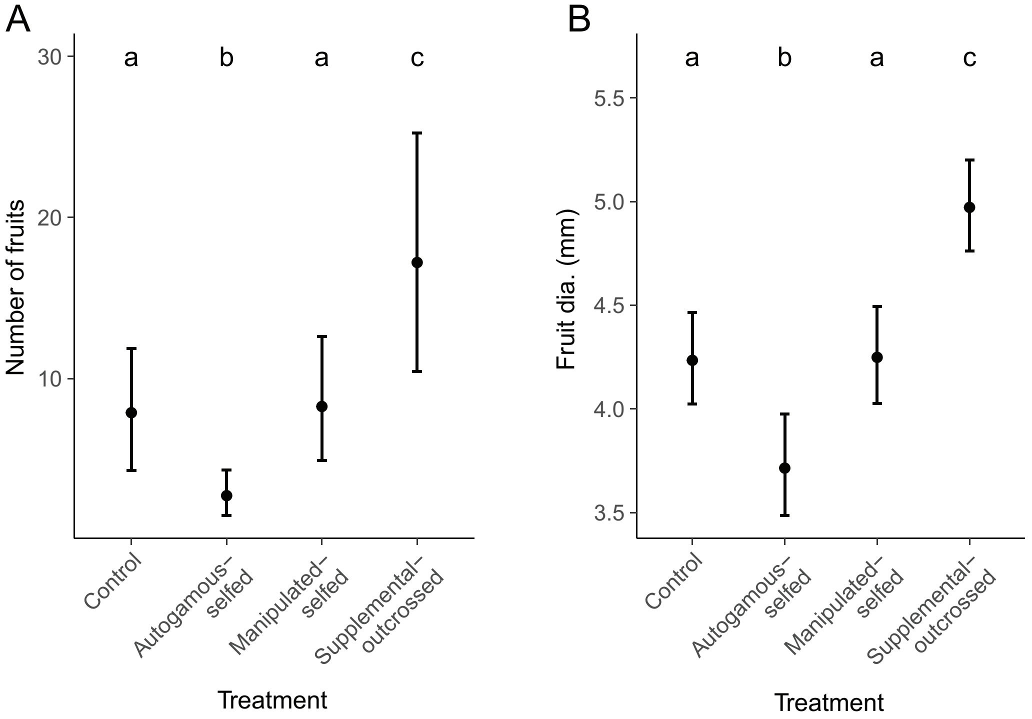

Figure 3: Mean and 95% bootstrap confidence interval for the number of fruits collected per plant for each treatment (*A*) and the diameter of fruits collected for each treatment (*B*). Different letters above the treatments indicate significantly different groups (unadjusted *P* values < .005).

tions and our high-speed videos, we saw pollen fly past the pollinator (video A5). Only with detailed experimentation and observations were we able to better understand the adaptive significance of explosive pollination—we realized that field-based observations did not allow us to see how much pollen actually hit the bee (because the bee's body often blocked the view). By triggering anthers manually and recording the location of projected pollen, we demonstrate that most of the pollen flies toward a bee collecting nectar at the center of the flower.

Though we cannot fully answer the question of the adaptive significance of explosive pollination in *K. latifolia*, we can provide evidence that is consistent with one hypothesis. Our results support the claim that *K. latifolia* is adapted to release pollen to large bees, which routinely touch the stigmas of the flowers as they probe for nectar. We therefore suggest that W. J. Beal (1867) was largely correct in hypothesizing that the elaborate catapult system targets bees to enhance cross pollination, though we did not disprove the hypothesis that the catapult mechanism enhances self-fertilization, which was proposed by Rothrock (1867). Past research points to different populations of *K. latifolia* relying on each of these methods of reproduction to different degrees. For instance, Rathcke and Real (1993) found that some pollen limited populations were capable of autogamous fertilization when pollinators had been excluded; we did find very low levels of seed set from this mechanism in our study, as well. In addition, several researchers (Jaynes 1988; Nagy et al. 1999) have suggested that aging *K. latifolia* flowers may launch pollen directly onto the stigma without the influence of a pollinator, as a way to assure reproduction by self-fertilization. In fact, Levri (2000) suggests that most uniparental breeding occurs without pollinators in some Virginia populations of *K. latifolia*. Other populations, however, show no pollen limitation, and thus delayed self-fertilization is unlikely (Rathcke 2003).

In our first experiment, we found that the catapult mechanism launches pollen with high speeds and accelerations. These quick movements may ensure that the pollen attaches to the pollinator while the insect is still on the flower. The high speed and acceleration also ensures that pollen will fly out of the anthers, rather than remaining stuck inside the anther locules. Our work reveals that mountain laurels have one of the fastest-moving floral parts recorded. The pollen of *K. latifolia* moves faster than the *Catasetum* orchid that fascinated Charles Darwin (1862*a*) and at a comparable speed, although lower acceleration, to *Catasetum canadensis* (Edwards et al. 2005). The release of pollen from *K. latifolia* has notable similarities to *C. canadensis*: both have filaments under tension, and they both fling pollen from the tips of the anthers. However, the anthers of *C. canadensis* are attached to the throwing arm (filament) by a hinge, which is a thin vascular strand connecting the anther to the filament tip (Edwards et al. 2005). The anthers, of *K. latifolia*, in contrast, do not have a hinge. This difference may help explain why *C. canadensis* accelerates pollen much faster than *K. latifolia*.

In our second experiment, we found that the pollen tends to fly toward the center of the flower (see fig. 2). This was not obvious when we recorded videos of insects visiting *K. latifolia* in the field. Because the pollen is launched in multiple clumps (that cover a wide fan of angles), we often observed some pollen fly past the pollinators' bodies (e.g., video A5). Indeed, our heat map suggests that when pollen does not cross the flower's central axis, it may occasionally miss the pollinators. Using our novel quantitative evaluation of where pollen travels after explosive dehiscence, we can conclude that if the pollinator is in the center of the flower foraging for nectar, most pollen will likely hit it.

From the heat map (fig. 2), we can predict what size insect will be the most effective pollinator of *K. latifolia* flowers. Notably, small pollinators may avoid being hit by the pollen (fig. 2). Small bees, for instance, may be too close to the floral nectary such that the pollen launches over the top of their bodies. Additionally, butterflies, with very long proboscises, can feed from the edge of the petals and thus be too far away from the center of the flower to be hit by pollen; the proboscis of a butterfly may also be too fragile to trigger the release of the anther. Overall, our heat map (fig. 2) is consistent with the hypothesis that *K. latifolia* is adapted for pollination by bees that are as big or bigger than foraging bumblebees (~15 mm in length; see Williams et al. 2014), which routinely trigger the anthers and touch the stigma of the flower during visits.

Our observations of pollinators and their behaviors in the field (the third part of our investigation) further corroborates our claim that the pollen catapult of *K. latifolia* is an adaptation that releases pollen to insects that are likely to transfer pollen to the stigma of another flower. In our observations, we recorded that only large bees triggered the anthers, though we did not observe any bees smaller than a honeybee visiting. Furthermore, bumblebees typically positioned their bodies in the center of the flower, near or touching the stigma. These observations agree with past studies, many of which suggest that *K. latifolia* relies almost exclusively on bumblebees (*Bombus* spp.; Jaynes 1988; Rathcke 1988; Real and Rathcke 1991; Rathcke and Real 1993; Nagy et al. 1999; Mathews and Collins 2014), though different regions likely have different *Bombus* species. Recent literature has found bumblebees (*Bombus bimaculatus*, *Bombus impatiens*), honeybees (*Apis mellifera*), and sweat bees (*Halictus confusus*) visiting *K. latifolia*. In our observations, we were able to identify *B. impatiens* and *A. mellifera* visiting *K. latifolia*. Past literature has also reported that pollinators visit *K. latifolia* only for nectar and not to collect pollen (Rathcke 1988, 2003), which is what we observed as well.

Our final experiment, which investigated the effect of pollinators on fruit size and number, suggests that pollinators play a critical role in sexual reproduction of *K. latifolia*. Most importantly, we found lower fruit number and small fruit size when pollinators were excluded from flowers (autogamous-selfed treatment) than when self-pollen was added to the stigma (manipulated-self treatment; fig. 3). Furthermore, we found that manipulated-selfed flowers had similar fruit set to control flowers. Our results indicate that these plants are self-compatible but that mechanical release of pollen by pollinators is important for effective transfer of pollen from anthers to stigmas, even for self-pollination. This result is consistent with some previous studies that also find that autogamous pollination does not significantly contribute to fruit production (Nagy et al. 1999). Yet others have reported that some populations of *K. latifolia* can self-fertilize without a pollinator (Rathcke and Real 1993) and that flowers may use delayed autogamous self-pollination for reproductive assurance (Levri 2000).

Finally, we found that supplemental-outcrossed *K. latifolia* had higher fruit set and larger fruits than any of the other treatments (fig. 3), suggesting that these plants are pollen limited. This could be due to several reasons. First, it could mean that pollinators are transferring ample pollen among flowers but that the pollinators are transferring self-pollen; this may cause *K. latifolia* to selectively abort selfed seeds over outcrossed seed (Levri 1998). Alternatively, the *K. latifolia* plants in our study may not have received enough pollinator visits to sufficiently transfer pollen among flowers. This may be due to a scarcity of pollinators at the location of our plants in the Arnold Arboretum, but other studies also indicate that *K. latifolia* is rarely visited by pollinators (Jaynes 1988; Real and Rathcke 1991; Rathcke and Real 1993). In our study, pollinators were so rare that we collected only 69 videos of insect visitors in ~20 h of filming, even though individual *K. latifolia* plants can have thousands of open flowers simultaneously (Rathcke and Real 1993). We hypothesize that *K. latifolia* in our study were pollen limited because flowers produce very little nectar (Jaynes 1988; Real and Rathcke 1991), and the nectar, and possibly pollen, is thought to be toxic to bees (Eckert 1955; Oertel 1980; Adler 2000). For these reasons, bumblebees may be more attracted to coflowering plants (Rathcke and Real 1993), of which there are many in the Arnold Arboretum. Though literature suggests bumblebees cannot detect the ecologically relevant levels of toxins in the nectar in a laboratory setting (Tiedeken et al. 2014), the poisonous nectar may still play a role in this system. Pollen limitation may suggest a selective advantage for explosive pollination. If pollinators are rare and if catapulting pollen increases the adherence of pollen to insects, then explosive pollination may increase rates of pollen transfer among flowers by pollinators.

Follow-up Experiments

Though our experiments help us understand the evolutionary significance of the pollen catapult in *Kalmia latifolia*, there are still many unanswered questions about pollination in this system. For instance, we have no information about the force required to trigger the pollen release and whether that force changes as flowers age. We also do not understand what role pollinator learning plays in this system—a follow-up experiment may investigate the extent to which bees learn to handle these flowers and how that affects fertilization rates. In addition, we do not know the nutritional value of pollen for the insects that visit *K. latifolia* or whether and how they collect pollen to feed their larvae. We also do not have enough data to describe where on the bee body pollen generally attaches—we were not able to obtain these data, because in many of the videos, bees' bodies blocked the camera's view of the pollen. The way that pollen flies out of the anther and the structure of the pollen clumps may enhance pollen attachment—for instance, the stringy pollen may act like a bola, where if one end of the pollen string hits the pollinator, then the rest of the string will wrap around and attach to the pollinator.

Conclusion

Our study combines a biomechanical evaluation of the *Kalmia latifolia* pollen catapult with an ecological evaluation of how explosive pollination contributes to reproduction. Our results support the hypothesis that the pollen catapult in *K. latifolia* is an adaptation for pollen dispensing—restricting pollen removal to certain insects (i.e., large bees) that are likely to transfer that pollen to the stigma of another flower. The explosive pollen release launches pollen toward the central axis of the flower, near the stigma, which is where the bumblebees are when they probe for nectar. We found that other insects rarely visit or trigger *K. latifolia* pollen catapults. Finally, we found that insect visitors play an important role in pollen movement, even in self-pollinated flowers. This work represents an integrative investigation of explosive pollination and provides a comprehensive understanding of how pollen is dispensed in this system.

Acknowledgments

We thank the Arnold Arboretum of Harvard University for providing access to the collections used in this study. We thank Arnold Arboretum collection curator Michael Dosmann and curatorial assistant Kathryn Richardson for their advice and insight. We thank Justin Dower and Andrew Clark for assisting with data collection. This material is based on work supported by the National Defense Science and Engineering Graduate Fellowship program, the Arnold Arboretum Sinnott Award, Air Force Office of Scientific Research grant FA9550-14-1-0398, and the University of Washington Data Science Grant from the Moore and Sloan Foundations and the Washington Research Foundation to C.M.S. It was also funded by a National Science Foundation grant (CAREER IOS-1253677) to S.A.C.

Literature Cited

Adler, L. S. 2000. The ecological significance of toxic nectar. Oikos 91:409–420.

Alemán, M., T. Figueroa-Fleming, Á. Etcheverry, S. Sühring, and P. Ortega-Baes. 2014. The explosive pollination mechanism in Papilionoideae (Leguminosae): an analysis with three *Desmodium* species. Plant Systematics and Evolution 300:177–186.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48. http://dx.doi.org/10.18637/jss.v067.i01.

Beal, W. 1867. Agency of insects in fertilizing plants. American Naturalist 1:254–260.

Bradski, G. 2000. The OpenCV library. Dr. Dobb's Journal of Software Tools. http://opencv.org/.

Darwin, C. 1862*a*. On the three remarkable sexual forms of *Catasetum tridentatum*, an orchid in the possession of the Linnean Society. Botanical Journal of the Linnean Society 6:151–157.

———. 1862*b*. On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effects of intercrossing. Cambridge University Press, Cambridge.

Ebel, F., A. Hagen, K. Puppe, H. Roth, and J. Röth. 1974. Beobachtungen über das bewegungsverhalten des Pollinariums von *Catasetum jimbriatum* Lindl. während abschuß, flug und landung. Flora 163:342–356.

Eckert, J. 1955. Bee diseases as factors in the life and behavior of the honeybee colony. Insectes Sociaux 2:187–194.

Edwards, J., D. Whitaker, S. Klionsky, and M. J. Laskowski. 2005. Botany: a record-breaking pollen catapult. Nature 435:164.

Fleming, T. F., and Á. V. Etcheverry. 2017. Comparing the efficiency of pollination mechanisms in Papilionoideae. Arthropod-Plant Interactions 11:273–283.

Friedman, W. E. 2009. The meaning of Darwin's "abominable mystery." American Journal of Botany 96:5–21.

Fulop, D. 2009. Biomechanics, pollination, and evolution of *Catasetum* (Catasetinae, Orchidaceae). PhD diss. Harvard University, Cambridge, MA.

Galen, C. 1999. Why do flowers vary? the functional ecology of variation in flower size and form within natural plant populations. BioScience 49:631–640.

Gould, S. J., and R. C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proceedings of the Royal Society B 205:581–598.

Hedrick, T. L. 2008. Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. Bioinspiration and Biomimetics 3:034001.

Hermann, P. M., and B. F. Palser. 2000. Stamen development in the Ericaceae. I. Anther wall, microsporogenesis, inversion, and appendages. American Journal of Botany 87:934–957.

Hurlbert, A. H., S. A. Hosoi, E. J. Temeles, and P. W. Ewald. 1996. Mobility of *Impatiens capensis* flowers: effect on pollen deposition and hummingbird foraging. Oecologia 105:243–246.

Jaynes, R. A. 1988. "Kalmia": the laurel book II. Timber, Portland, OR.

Levri, M. A. 1998. The effect of timing of pollination on the mating system and fitness of *Kalmia latifolia* (Ericaceae). American Journal of Botany 85:1626–1630.

———. 2000. A measure of the various modes of inbreeding in *Kalmia latifolia*. Annals of Botany 86:415–420.

Mathews, K., and B. Collins. 2014. Plant and pollinator communities of high elevation rock outcrops. Natural Areas Journal 34:300–309.

Miller, R. B. 1981. Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. Evolution 35:763–774.

Nagy, E. S., L. Strong, and L. F. Galloway. 1999. Contribution of delayed autonomous selfing to reproductive success in Mountain Laurel, *Kalmia latifolia* (Ericaceae). American Midland Naturalist 142:39–46.

Nicholson, C. C., J. W. Bales, J. E. Palmer-Fortune, and R. G. Nicholson. 2008. Darwin's bee-trap: the kinetics of *Catasetum*, a new world orchid. Plant Signaling and Behavior 3:19–23.

Niklas, K. J. 1992. Plant biomechanics: an engineering approach to plant form and function. University of Chicago Press, Chicago.

Nilsson, L. A. 1988. The evolution of flowers with deep corolla tubes. Nature 334:147–149.

Nimmo, J. R., P. M. Hermann, M. Kirkham, and E. R. Landa. 2014. Pollen dispersal by catapult: experiments of Lyman J. Briggs on the flower of mountain laurel. Physics in Perspective 16:371–389.

Oertel, E. 1980. Nectar and pollen plants. Agriculture Handbook 335: 16–24.

Pischtschan, E., and R. Claßen-Bockhoff. 2008. Setting-up tension in the style of Marantaceae. Plant Biology 10:441–450.

Poppinga, S., K. Koch, H. F. Bohn, and W. Barthlott. 2010. Comparative and functional morphology of hierarchically structured anti-adhesive surfaces in carnivorous plants and kettle trap flowers. Functional Plant Biology 37:952–961.

Python Software Foundation. 2016. Python language reference. Version 2.7.13. https://www.python.org/.

Raju, A., and C. S. Reddi. 1995. Explosive pollen release and pollination in flowering plants. Proceedings of the Indian National Science Academy 61:323–332.

Ramachandran, P., and G. Varoquaux. 2011. Mayavi: 3D visualization of scientific data. Computing in Science and Engineering 13: 40–51.

Rathcke, B. 1988. Flowering phenologies in a shrub community: competition and constraints. Journal of Ecology 76:975–994.

———. 2003. Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). American Journal of Botany 90:1328–1332.

Rathcke, B., and L. Real. 1993. Autogamy and inbreeding depression in mountain laurel, *Kalmia latifolia* (Ericaceae). American Journal of Botany 80:143–146.

R Development Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Real, L. A., and B. J. Rathcke. 1991. Individual variation in nectar production and its effect on fitness in *Kalmia latifolia*. Ecology 72:149–155.

Rothrock, J. 1867. The fertilization of flowering plants. American Naturalist 1:64–72.

Sarwar, A. G., and H. Takahashi. 2012. Pollen morphology of *Kalmia* L. (Phyllodoceae, Ericaceae) and its taxonomic significance. Bangladesh Journal of Plant Taxonomy 19:123.

Schemske, D. W., and C. C. Horvitz. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science 225:519–521.

Solomon, A., and S. P. Rao. 2006. Explosive pollen release and pollination as a function of nectar-feeding activity of certain bees in the biodiesel plant, *Pongamia pinnata* (L.) Pierre (Fabaceae). Current Science 90:960–967.

Steen, R., O. Aase, and A. L. Thorsdatter. 2011. Portable digital video surveillance system for monitoring flower-visiting bumblebees. Journal of Pollination Ecology 5:90–94.

Switzer, C. M., S. A. Combes, and R. Hopkins. 2018. Data from: Dispensing pollen via catapult: explosive pollen release in mountain laurel (*Kalmia latifolia*). American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.65sd4.

Taylor, P. E., G. Card, J. House, M. H. Dickinson, and R. C. Flagan. 2006. High-speed pollen release in the white mulberry tree, *Morus alba* l. Sexual Plant Reproduction 19:19–24.

Temeles, E. J., and A. G. Rankin. 2000. Effect of the lower lip of *Monarda didyma* on pollen removal by hummingbirds. Canadian Journal of Botany 78:1164–1168.

Tiedeken, E. J., J. C. Stout, P. C. Stevenson, and G. A. Wright. 2014. Bumblebees are not deterred by ecologically relevant concentrations of nectar toxins. Journal of Experimental Biology 217:1620–1625.

USDA, NRCS. 2017. The PLANTS database. National Plant Data Team, Greensboro, NC. http://plants.usda.gov.

Williams, P. H., R. W. Thorp, L. L. Richardson, and S. R. Colla. 2014. Bumble bees of North America: an identification guide. Princeton University Press, Princeton, NJ.

Wilson, P. 1995. Selection for pollination success and the mechanical fit of *Impatiens* flowers around bumblebee bodies. Biological Journal of the Linnean Society 55:354–383.

Natural History Editor: Mark A. McPeek