

DOES LACK OF POLLINATION EXTEND FLOWER LIFE?

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Abstract—Across angiosperm species, the longevity of individual flowers can range from fixed to highly plastic. The orchid family is noteworthy for frequent reports of species in which flower lifespans are greatly prolonged if flowers are not pollinated. Less dramatic cases of pollination-induced senescence of anthesis have been reported for various species in other families, but such reports are scattered. Frequently, such findings are peripheral components of more general pollination studies. Because pollination-dependent plasticity can ameliorate phenological dislocations between plants and pollinators, it is worthwhile to conduct systematic surveys of its magnitude and taxonomic distribution. As a start, we report a set of experiments comparing the active lifespans of pollinated flowers to those of unpollinated controls in a set of nine species from a local subalpine flora. In all species, unpollinated flowers had longer mean times of receptiveness than pollinated ones, although the differences in means were often small. Three species exhibited significantly extended floral longevity in the absence of pollination.

Keywords: Plasticity, anthesis, floral longevity, pollination-induced senescence, Rocky Mountain Biological Laboratory

INTRODUCTION

The longevity of individual flowers varies across plant species; some of the variation is species-specific and genetically determined (Primack 1985; Stratton 1989), and some of it arises from plastic responses to immediate conditions. Here we are concerned with one potential plastic response, the extension of flower life as a response to lack of pollination. Such extension is particularly relevant in the context of recent concerns that climate change can cause phenological mismatches between plants and their pollinators (Kudo et al. 2004; Memmott et al. 2007; McKinney et al. 2012). As environmental conditions change, plants and pollinators may respond to different cues and emerge at different times, leading to pollination deficits for plants and food shortages for pollinators. The detrimental effects of phenological mismatches can be ameliorated, however, if plants can prolong the lifespans of flowers that have not been pollinated. In Colorado subalpine meadow communities, for example, Forrest & Thomson (2011, p. 487) argued that plant-pollinator dislocations in time are likely to be “quantitative effects... rather than... complete decoupling of formerly interacting organisms.” In such situations, the ability of flowers to prolong their lifespans by even a few days might substantially increase the probability of receiving pollinating visits.

Floral longevity, or the length of time that a flower is open and functional (Ashman & Schoen 1994), has been treated as a resource allocation strategy in which the maintenance of flowers diverts resources from other reproductive or vegetative functions (Schoen & Ashman 1995; Ashman & Schoen 1997). Increased floral longevity is likely to increase plant reproductive success through

prolonged pollen and stigma presentation (Thomson & Barrett 1981; Lloyd & Yates 1982; Galen et al. 1986; Harder & Thomson 1989), but it also exacts significant carbon and water costs through nectar production, respiration, and transpiration (Ashman & Schoen 1997). Thus, floral longevity can be viewed as a trade-off between fitness accrual through reproduction and the costs of floral maintenance.

Consequences of this trade-off could be particularly dramatic in many orchid species, where pollination can trigger rapid senescence of flowers that would stay receptive much longer if unpollinated (van Doorn 1997). Pollination-induced senescence has been reported in diverse genera such as *Leporella* and *Caladenia* (Peakall 1989), *Encyclia* (Ackerman 1989), *Cypripedium* (Primack & Hall 1990), *Cleistes* (Gregg 1991), *Calypso* (Proctor & Harder 1995), and *Myrmecophila* (Parra-Tabla et al. 2009). The effects of reproductive activity on floral longevity are not restricted to female function: in *Chloraea alpina*, pollinia removal, in addition to deposition, shortened the longevity of unpollinated flowers (Clayton & Aizen 1996). Similarly, both pollinia removal and deposition induced senescence in *Cattleya porcia* in an ethylene-dependent process (Strauss & Arditti 1984).

Orchids are an extreme case. Nevertheless, in other families, floral longevity does respond plastically to the completion of male and/or female function. Effects of pollen deposition on floral lifespan have been reported in numerous families, including Onagraceae (Addicott & Lynch 1955; Ashman & Schoen 1997), Caryophyllaceae (Nichols 1971; Motten 1986), Solanaceae (Gilissen 1976, 1977), Plantaginaceae (Stead & Moore 1979, 1983), Campanulaceae (Devlin & Stephenson 1984, 1985; Richardson & Stephenson 1989; Evanhoe & Galloway 2002), Liliaceae (Schemske et al. 1978; Motten 1983, 1986; Ishii & Sakai 2000), Portulacaceae (Motten 1986; Aizen

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FIGURE 1. Photos of nine animal-pollinated species surveyed for pollination-induced senescence in and near the Rocky Mountain Biological Laboratory, Gothic, Colorado. Top row, left to right: *Mertensia fusiformis* (Boraginaceae), *Delphinium barbeyi*, *Aconitum columbianum* (Ranunculaceae); second row, left to right: *Ipomopsis aggregata* (Polemoniaceae), *Vicia americana* (Fabaceae), *Chamerion angustifolium* (Onagraceae); third row, left to right: *Sidalcea candida* (Malvaceae), *Gentianopsis detonsa* (Gentianaceae), *Campanula rotundifolia* (Campanulaceae). All photos by H. F. Fung.

1993), Brassicaceae (Motten 1986; Preston 1991), Gentianaceae (Webb & Littleton 1987), and Ericaceae (Rathcke 1988a, 1988b; Blair & Wolfe 2007). In *Lobelia cardinalis* and *Campanula rapunculoides*, pollen removal and deposition shortened the duration of the staminate and pistillate phases respectively (Devlin & Stephenson 1984; Richardson & Stephenson 1989). Interestingly, pollen removal, but not deposition, accelerated senescence in *Brassica napus* (Bell & Cresswell 1998).

To broaden the study of pollination-induced senescence and to further explore its implications for phenological mismatch, we surveyed nine plant species (Fig. 1) at or near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA. A great deal of pollination research, including the previously cited study of phenological

dislocation by Forrest and Thomson (2011), has been conducted near this field station. In addition, the mating systems and pollinator faunas of most species at Gothic are well-characterized. The species in this study were chosen for convenience: most are abundant, and they produce large, tractable flowers that are characteristically visited by bees or hummingbirds. Using a series of controlled hand-pollinations, we examined whether plants in this subalpine community can prolong the lifespan of unpollinated flowers.

MATERIALS AND METHODS

We sampled the nine species at sites within and near the RMBL (38.96° N, 106.99° W, 2900 m asl) from June to August 2016. To minimize environmental variation,

individuals of each species were sampled at one site. Prior to flower opening, we covered plants with polyolefin drawstring bags or sand-bag style exclusion bags (Thomson et al. 2011) to exclude flower visitors.

We marked the pedicels of two flowers, paired for size, floral age, and position on plant, with felt-tipped markers. We chose species-specific markers of floral age that could be scored by gross inspection, such as bud break, stylar exertion, wilting, colour changes, and abscission (see Appendix 1). These easily scored characteristics may not precisely delimit the onset and cessation of flower functions, but we consider them appropriate for detecting differences in floral lifespan in our paired design.

Within pairs, flowers were randomly assigned to experimental or control treatments. Once mature and receptive, experimental flowers were hand-pollinated with Microbrush® applicators (Microbrush International; Grafton, Wisconsin, USA) bearing a mixture of fresh pollen from other plants in the vicinity. We attempted to apply as much pollen as the stigmas could retain. Control flowers were not pollinated, but were manipulated in the same way as experimental flowers with clean applicators. To ensure successful hand pollination, we pollinated each flower twice, on consecutive days. An exception was *Campanula rotundifolia*, which was hand pollinated three times. Criteria for stigma receptivity are provided in the Supplementary Data.

We checked individuals only once a day. Although more frequent checks could have produced finer-grained data, we compromised so as to be able to score more species and more replicates. We scored flowers for a range of floral age indicators, including extent of anther dehiscence, perianth colour, openness or accessibility to interior (Olesen et al. 2007), degree of wilting, and corolla abscission (details in Supplementary Data). Our response variable, floral lifespan, was the number of days to senescence from first hand-pollination.

Data analysis

We used one-tailed exact Wilcoxon signed rank tests to determine whether anthesis was prolonged in unpollinated flowers. Analyses were performed using R v. 3.1.2 (R Foundation for Statistical Computing 2014). Data are expressed as means \pm standard deviation.

RESULTS

The extension of flower life in unpollinated flowers varied across species (Fig. 2). Based on single Wilcoxon tests, the following species showed evidence of pollination-induced senescence (mean paired differences, control - treatment): *Mertensia fusiformis* (0.67 ± 0.87 days, $V = 77$, $P = 0.011$), *Chamerion angustifolium* (0.58 ± 1.82 days, $V = 165$, $P = 0.039$), and *Gentianopsis detonsa* (2.71 ± 1.93 days, $V = 253$, $P < 0.0001$). The remaining species did not: *Delphinium barbeyi* (0.07 ± 1.36 days, $V = 150$, $P = 0.58$), *Ipomopsis aggregata* (2.64 ± 4.36 days, $V = 161$, $P = 0.14$), *Vicia americana* (0.07 ± 2.71 days, $V = 90$, $P = 0.31$), *Aconitum columbianum* (0.56 ± 2.17 days, $V = 147$,

$P = 0.058$), *Sidalcea candida* (0.26 ± 0.62 days, $V = 52$, $P = 0.19$), and *Campanula rotundifolia* (0.33 ± 2.09 days, $V = 39$, $P = 0.61$). Despite the lack of significance for the majority of the species, it is worth noting that control flowers had longer estimated mean lifetimes in all nine species, which is itself a highly significant pattern by sign test ($P = 0.0039$).

DISCUSSION

This study represents one of the few attempts to explore pollination-mediated senescence across species within a local community. Of the nine species surveyed in and near the RMBL, three showed individually significant evidence of pollination-induced senescence (Fig. 2). Overall, unpollinated flowers did tend to last longer, but for the majority of species the differences were too small and inconsistent to overcome the substantial variation within treatments. In further studies, larger sample sizes would be desirable. Our results suggest that species vary in their ability to prolong the lifespan of unpollinated flowers, a finding that is consistent with other broad surveys of floral longevity. In a study of the spring wildflower community in North Carolina, pollen deposition triggered floral senescence in five of eight species (Motten 1986). Likewise, pollination-induced senescence was observed in four of six shrub species in The Great Swamp, Rhode Island, USA (Rathcke 1988b). Evidently, meaningful plasticity in floral lifespan is common but not universal.

Flowers of *Gentianopsis detonsa* responded to pollination in a similar fashion to two closely related species, *Gentiana saxosa* and *G. serotina*. In addition to changes in colour and turgor, pollination caused the corollas of these species to close (H. F. Fung, pers. obs.; Webb & Littleton 1987), which may protect the developing ovary from predators and subsequent pollinations (Webb & Littleton 1987). Thus, corolla closure may be a common response to pollination among the gentians. Interestingly, we did not find evidence of pollination-induced senescence in *Campanula rotundifolia* (Fig. 2), in contrast to studies of *C. rapunculoides* (Richardson & Stephenson 1989) and *C. americana* (Evanhoe & Galloway 2002).

From a resource allocation perspective, pollination-induced senescence offers a way in which plants can plastically optimize the trade-off between reproductive success and the costs of maintaining flowers (Primack 1985; Harrison & Arditti 1976; Ishii & Sakai 2000). Through pollination-induced senescence, plants can direct their resources toward maintaining unpollinated flowers, and in doing so, reduce the likelihood that plants and their pollinators are phenologically mismatched. The findings presented here and elsewhere indicate that certain plants can prolong the lifespans of unpollinated flowers, but it is unclear that this is prevalent enough to ameliorate phenological asynchrony substantially. After all, pollination had no effect on floral longevity in the majority of plant species surveyed in the present study.

There are several reasons why floral longevity may not evolve to respond plastically to pollination. First, the trade-

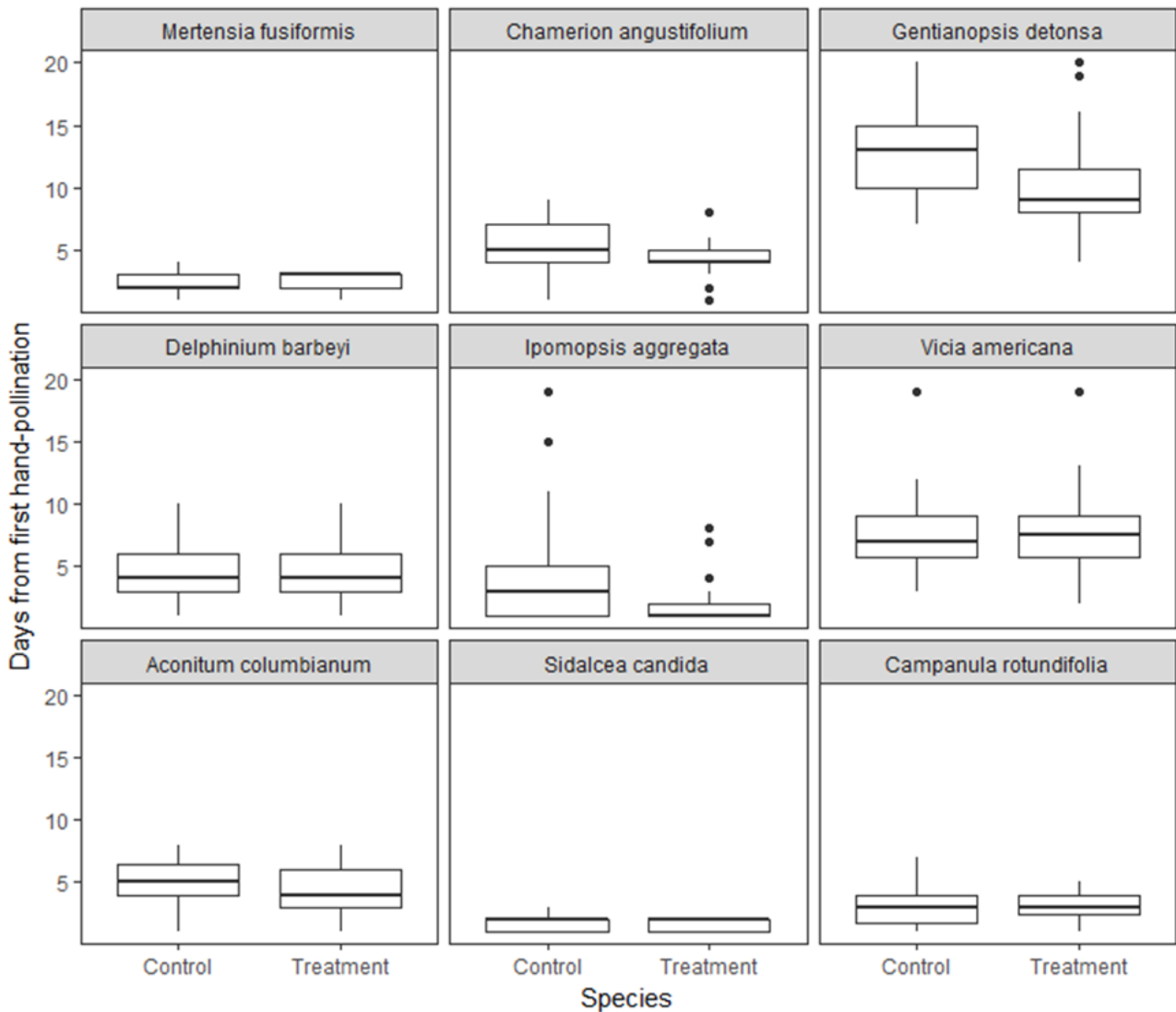


FIGURE 2. Floral longevity of nine plant species in or near the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA. Experimental flowers were hand-pollinated once receptive, while control flowers were not pollinated. Sample sizes, in pairs, for the species, in order from left to right, top to bottom: 13, 25, 19, 29, 28, 28, 27, 26, 18.

off between reproductive success and maintenance costs may not be as pronounced in plants that continue to grow and photosynthesize after reproduction (Ashman & Schoen 1997). In the snow buttercup *Ranunculus adoneus*, for example, flowers accounted for a significant proportion of carbon assimilation (Galen et al. 1993). Similarly, in *Ambrosia trifida* L., reproductive structures contributed 41% and 57% of the carbon required to construct male and female inflorescences respectively (Bazzaz & Carlson 1979). Moreover, increased photosynthesis in adjacent leaves may compensate for some of the costs of floral maintenance (Gifford & Evans 1981; Lehtilä & Syrjänen 1995; Ashman & Schoen 1997). Such cases are more complicated than the simple concept of allocation from a fixed pool of resources.

Second, plants may continue to maintain pollinated flowers to increase floral display size and to facilitate long-distance attraction of pollinators (Primack 1985; reviewed by Snow et al. 1996; Evanhoe & Galloway 2002). As display size increases, pollinator visitation tends to increase,

increasing pollen export and receipt. Large displays, however, can represent a significant drain on resources (Evanhoe & Galloway 2002) and can increase the rate of geitonogamy (Barrett & Harder 1996; Snow et al. 1996).

Finally, flowers can contribute to male reproductive success even after they are pollinated (Primack 1985; Ishii & Sakai 2000). In *Erythronium japonicum*, for example, floral longevity was nearly constant in flowers pollinated between days one and 12 of anthesis (Ishii & Sakai 2000). This 12-day period was subsequently shown to be necessary for *E. japonicum* flowers to shed the majority of their pollen (Ishii & Sakai 2000). Based on these results, Ishii & Sakai (2000) proposed that flowers have a genetically determined minimum longevity that functions to facilitate male reproduction.

This 'minimum longevity' hypothesis yields the following prediction: pollination-induced senescence should be more common in species in which senescence in response

to pollination does not interfere with pollen dispersal, as in protandrous species (Ashman & Schoen 1996; Ishii & Sakai 2000; Evanhoe & Galloway 2002). There is some evidence that protandrous species are more likely to show pollination-induced senescence. In a survey of spring wildflowers in North Carolina, Motten (1986) found that weakly protogynous and protandrous species were more likely to senesce in response to pollen deposition than protogynous species. In the present study, however, pollination significantly accelerated senescence in only two of seven protandrous species (Fig. 2).

To address this apparent discrepancy, it is important to recognize that floral longevity may be governed by the pollination status at the plant level, as opposed to the flower level. In other words, plants in which the majority of ovules have been fertilized may present shorter-lived flowers than their unpollinated counterparts. As a result, among-plant comparisons may reveal instances of pollination-induced senescence that were not detected at the within-plant level, as in the cases of *Delphinium*, *Ipomopsis*, *Vicia*, *Aconitum*, *Sidalcea*, and *Campanula* (Fig. 2).

To conclude, we observed significant pollination-induced senescence in three out of nine study species in and near the RMBL, but the magnitudes of those effects were small-half a day to a few days. Regarding our motivating question of phenological dislocation, plastic extensions of floral lifespan may frequently have trivial effects. Still, species of particular interest should be examined individually.

APPENDICES

Additional supporting information may be found at the end of this article:

APPENDIX I. Criteria for measuring floral age, stigma receptivity, and senescence.

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APPENDICES

Appendix I. Criteria for measuring floral age, stigma receptivity, and senescence.

Species	Floral age	Stigma receptivity	Senescence
<i>Mertensia fusiformis</i> Boraginaceae	- Extent of dehiscence - Openness or accessibility to interior (Olesen et al. 2007)	Assumed flowers were receptive when open Hand-pollinated on days 2 & 3 of opening	Corolla abscission
<i>Delphinium barbeyi</i> Ranunculaceae	- Extent of dehiscence - Openness	Day 3 of anthesis (Luo et al. 2014)	Corolla abscission
<i>Aconitum columbianum</i> Ranunculaceae	- Extent of dehiscence - Openness - Corolla size	Day 4 of anthesis (Luo et al. 2014)	Corolla abscission
<i>Ipomopsis aggregata</i> Polemoniaceae	- Extent of dehiscence - Openness - Style length - Corolla length	When stigma trifurcates (Pleasants 1983)	Corolla abscission
<i>Vicia americana</i> Fabaceae	- Extent of dehiscence - Openness - Corolla colour	Assumed flowers were receptive when open Hand-pollinated on days 2 & 3 of opening	Corolla abscission
<i>Chamerion angustifolium</i> Onagraceae	- Extent of dehiscence - Openness	When stigma lobes are reflexed (Galen & Plowright 1985)	Corolla abscission
<i>Sidalcea candida</i> Malvaceae	- Extent of dehiscence - Openness - Style length	When stigma is exposed	Wilting and/or closure
<i>Gentianopsis detonsa</i> Gentianaceae	- Extent of dehiscence - Openness - Corolla size	When stigma is open	Corolla bleaching
<i>Campanula rotundifolia</i> Campanulaceae	- Amount of pollen on style - Openness	When stigma trifurcates (Nyman 1992)	Wilting

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