Sit-and-wait pollination in the spring flowering woodland plant, *Trillium grandiflorum*

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Abstract—In animal-pollinated plants, reproductive success is commonly limited by pollen availability, which can occur in environments where pollinator activity is scarce or variable. Extended floral longevity to maximize a plant's access to pollinators may be an adaptation to such uncertain pollination environments. Here, we investigated the effects of flower exposure time to pollinators on female fertility (fruit and seed set) in the bee-pollinated woodland herb *Trillium grandiflorum*, a species with long-lived flowers (~17-21 d) that blooms in early spring when pollinator activity is often variable. We experimentally exposed flowers to pollinators for different amounts of time to determine the extent to which floral longevity influenced reproductive success. The amount of time that flowers were exposed to pollinators significantly increased fruit set and seed set per flower, but not seed set per fruit. Our results provide experimental evidence that long floral life spans may function as a 'sit-and-wait' pollination strategy to increase the amount of exposure time to pollinators and promote seed set in the unpredictable pollination environments often experienced by early spring ephemerals. In large populations with infrequent pollinator visitation, as commonly occurs in *T. grandiflorum*, pollination may be a largely stochastic process.

Keywords: Flower longevity, pollinator exposure, pollen limitation, stochastic pollination environment, bee pollination

Introduction

The reproductive success of plants is often limited by pollen availability (reviewed in Burd 1994; Larson & Barrett 2000; Ashman et al. 2004; Knight et al. 2005). This occurs when the quantity and/or quality of pollen that a plant receives during pollination is insufficient to fertilize available ovules, resulting in a reduction in fruit and/or seed production (Burd 1995; Aizen & Harder 2007). The fitness consequences of pollen limitation in variable pollination environments are diverse, and chronic pollen limitation can affect the evolution of a range of life history and reproductive traits in plant populations (Haig & Westoby 1988; Ashman et al. 2004; Morgan et al. 2005; Porcher & Lande 2005; Harder & Aizen 2010). Extended floral longevity is one trait that can increase opportunities for pollinators to visit flowers and buffer fertility in stochastic environments with few pollinators (Kerner von Marilaun 1895; Primack 1985; Ashman & Schoen 1994; Charnov 1996). Theoretical models have examined the factors influencing optimal floral longevities (Ashman & Schoen 1994; Schoen & Ashman 1995), but relatively few studies have experimentally examined the direct consequences of floral longevities on female reproductive success (but see Ashman & Schoen 1997; Rathcke 2003; Alonso 2004).

Early spring-flowering woodland herbs in temperate climates often persist in variable pollination environments characterized by low temperatures and uncertain pollinator service (Schemske et al. 1978; Motten 1986; Barrett &

Helenurm 1987). *Trillium grandiflorum* (Melanthiaceae) is a self-incompatible woodland herb in eastern North America that depends on bee pollinators (mostly *Bombus* spp.) for pollen-transfer (Broyles et al. 1997; Sage et al. 2001) and populations are often pollen limited (Lubbers & Lechowicz 1989; Wright & Barrett 1999; Irwin 2000; Griffin & Barrett 2002). Flowers of *T. grandiflorum* are exceptionally long-lived (17-21 d; Ashman & Schoen 1996; Sage et al. 2001) and the goal of our study was to investigate if long floral lifespan acts as a 'sit-and-wait' strategy (e.g. Schoener 1971) to increase the chances of a pollinator visit and female reproductive success. To test this hypothesis, we experimentally manipulated the exposure time of flowers to pollinators as a proxy for floral lifespan. Our study had three objectives: (1) To characterize the pollination environment of *T. grandiflorum* by quantifying pollinator activity and ambient temperature during the flowering season, (2) To determine the extent to which fruit and seed set per fruit in *T. grandiflorum* may be pollen limited, and (3) To investigate the importance of exposure time to pollinators on these components of reproductive success.

Materials and methods

Study organism and site

Trillium grandiflorum is a perennial, non-clonal herb that occurs in the deciduous forest understory of eastern North America (Case & Case 1997). Populations flower for approximately three weeks in early spring and are pollinated largely by pollen-foraging bees (Case & Case 1997; Irwin 2000; Sage et al. 2001). Flowers produce no nectar and are weakly protandrous (Irwin 2000; S.C.H. Barrett unpubl.

observations). Each plant produces a single flower with on average 25.8 ± SEM 0.9 ovules, $n = 142$, at our study site. We performed our experiments between May and June 2005 at a large population of several thousand plants covering approximately 1 ha at the Koffler Scientific Reserve at Joker's Hill in southern Ontario, Canada (44.03°N, 79.53°W; http://www.zoo.utoronto.ca/jokershill/jh.html). At this location, *T. grandiflorum* is self-incompatible and predominantly outcrossing ($t = 0.68 \pm 0.09$, Sage et al. 2001), as has been reported in other populations of the species (Broyles et al. 1997; Kalisz et al 1999).

Pollination environment

To estimate pollinator activity, we recorded the number of bees that were active during flowering within the study population. Queens of *Bombus* spp. are the primary pollinator of *Trillium grandiflorum* at our study site (Griffin & Barrett 2002) although *Andrena carlini* Cockerell was also observed visiting flowers. We recorded the number of bee flights on six transects (~100 m each) in the population for a total of 44.5 h over 18 d throughout the flowering season, an average of 2.5 hours per day. We conducted pollinator observations between mid-morning and mid-afternoon when bee activity is highest. A bee flight was considered to be any time we observed an individual flying within the population, not necessarily visiting flowers. We were careful not to recount individual pollinators; this was possible due to the low pollinator activity at the site (0.08 visits/h, see also Irwin 2000). We therefore consider our observations to reflect the potential for pollination and we assume that the amount of effective pollination is likely to increase with the number of bees active in the community.

We obtained average daily temperatures (°C) from the nearest Environment Canada weather station located at Buttonville, Ontario (43.52°N, 79.22°W), ~20 km east of the study site. We evaluated the relationships between temperature, bee abundance and the number of days since the beginning of flowering (anthesis). We used Pearson partial correlations (ρ) of temperature and pollinator abundance while controlling for days since anthesis to account for the potential non-independence of daily temperature estimates (temporal autocorrelation).

Measurement of pollen limitation

We performed a pollen supplementation experiment on *T. grandiflorum* to investigate if female fertility at our site was pollen limited. We selected 40 plants of similar size and randomly assigned each one to either an open- or hand-pollination treatment. For the hand-pollinated treatment, we applied fresh pollen from three different donor plants that were located more than 1 m but less than 10 m away from the focal plant to limit effects of biparental inbreeding. Pollen was applied in a single application to stigmas and flowers began to senesce and turn pale pink several days after supplemental pollination (E. S. Darling and S.C.H. Barrett, pers. obs.). Open-pollinated plants were left unmanipulated for the entire duration of the flowering season, which in 2005 was 25 days.

In mid-July, we determined whether each flower had set fruit and collected all fruits. We counted the number of mature seeds, shrunken seeds and apparently unfertilized ovules under a dissecting microscope (see Griffin & Barrett 2002). We calculated fruit set as the proportion of individual plants producing a fruit with mature seeds. Seed set (the proportion of mature seeds out of the total number of ovules) was calculated per fruit and per flower. Seed set per fruit considered only the subset of flowers that had set fruit with at least one mature seed, while seed set per flower considered all flowers in the sample, regardless of whether they produced seed. We used Student *t*-tests to compare female fertility between open-pollinated and hand-pollinated treatments.

Determinants of female reproductive success

We tagged 120 individuals when flowers were in bud and recorded the approximate start date of anthesis (the beginning of flowering) for each plant, judged by when flowers were fully open and pollen was visible on anthers. At anthesis, we measured the height of the flower from the ground (hereafter plant height) and the density of reproductive individuals in a 1m² area around the focal plant (neighborhood flower density). We used plant height as an indicator of investment in floral attractive structures since this measure has been shown to correlate with both flower biomass ($r = 0.51$, $P < 0.0001$) and pollen number ($r = 0.93$, $P < 0.0001$) in this population (Wright & Barrett 1999).

To evaluate the extent to which floral longevity contributes towards reproductive success, we manipulated the amount of time that *T. grandiflorum* flowers were exposed to pollinators. Prior to flowering, we randomly assigned the 120 tagged individuals to nine exposure times ranging between two and 25 days. At the end of each exposure time, flowers were emasculated and covered with bridal veil bags to prevent further pollination. We treated exposure time as a proxy for flower lifespan and we assume stigmas are receptive during the entire period that flowers are exposed to pollinators. Previous studies in the same population support this assumption and found no effect of pollination timing or flower age on fruit and seed set (Griffin & Barrett 2002). We calculated pollinator availability for each flower as the mean number of observed pollinators day⁻¹ during the days the flower was exposed to pollinators. We collected mature fruits in mid-July 2005 and calculated fruit set and seed set per fruit (seed set for the subset of flowers with at least one mature seed) and per flower (seed set for all flowers) as described above.

We evaluated the relationship between exposure time and three measures of female reproductive success, fruit set, seed set per fruit and seed set per flower using linear relationships; exposure time was log-transformed prior to analysis. We evaluated the effect of plant height, neighbourhood flower density, pollinator availability and exposure time on seed set per flower using a general linear model (GLM) with a quasibinomial distribution to account for overdispersion (Zuur et al. 2009). We checked for collinearity between predictor variables using variance inflation factors (VIFs); the four predictor variables had

VIFs < 2 and were all included in the full model (Zuur et al. 2010). Model diagnostics were performed by visually evaluating the distribution of the residuals. All analyses were performed in R (version 2.11.1; www.r-project.org).

RESULTS

Pollination environment

Flowering of *Trillium grandiflorum* in 2005 in our study population began on 10 May and continued until 4 June. Bees were observed on ten days during the flowering season with none observed during the first week of flowering (10 - 17 May). The average daily number of bees observed was 2.22 ± 2.85 (mean ± SEM) and a total of 40 separate observations of *Bombus* queens and *Andrena carlini* were made in the population during the flowering season. The vast majority of bee flights did not include flower visits and we recorded only four visits to flowers in which pollination is likely to have occurred over 44.5 hours of observation.

There was an increasing trend in mean daily temperature and bee observations throughout the flowering season (Fig. 1). Pollinator abundance was correlated with daily temperature ($r = 0.70$, $P < 0.01$) and the number of days since the beginning of flowering ($r = 0.65$, $P < 0.01$). After accounting for temporal autocorrelation across the flowering
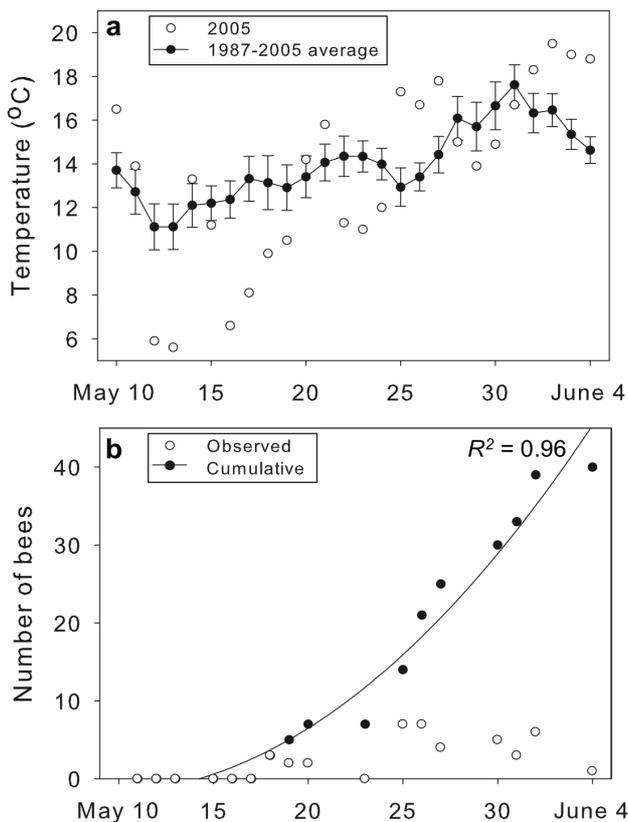
FIGURE 1. Conditions that favour pollination of *Trillium grandiflorum* increase throughout the flowering season. (a) Daily temperature (°C) in 2005 (open circles) and historical daily temperatures (mean ± SEM) between 1987 and 2005 (filled circles). (b) The number of observed (open circles) and cumulative (filled circles) bee pollinators.
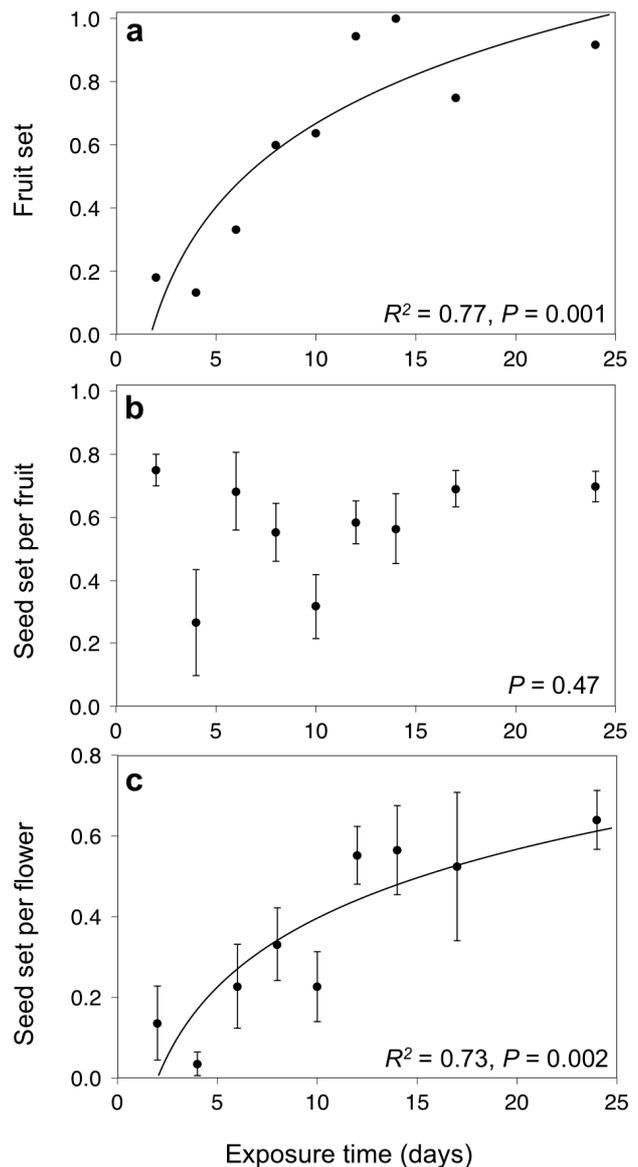
FIGURE 2. Reproductive success increases with exposure time in *Trillium grandiflorum*. There was a significant positive relationship between exposure time and (a) fruit set, and (c) mean seed set per flower, but not (b) mean seed set per fruit. Seed set values are based on seed:ovule ratios and are averages of individual plants within each exposure group treatment ± SEM. Sample sizes at exposure times of 2, 4, 6, 8, 10, 12, 14, 17, 24 d were 2, 2, 4, 9, 9, 17, 6, 5, 11 ($n = 65$) for fruits, and 11, 15, 12, 15, 13, 18, 6, 6, 12 ($n = 108$) for flowers, respectively.

season (number of days since anthesis), pollinator abundance remained significantly correlated with temperature ($\rho = 0.54$, $P = 0.03$). There was no correlation between pollinator abundance and days from the start of the flowering season after the effect of temperature was removed ($\rho = 0.39$, $P = 0.14$).

Pollen limitation

Despite low pollinator activity, supplemental hand pollinations provided no evidence that plants of *T. grandiflorum* were pollen limited in 2005. There were no significance differences between the open-pollinated (OP)

and hand-pollinated (HP) treatments in fruit set (OP = 0.79, HP = 1.0; $t_{df=24}$ = 1.08, P = 0.86), seed set per fruit (mean ± SEM: OP = 0.70 ± 0.05, HP = 0.62 ± 0.06; $t_{df=24}$ = -0.571, P = 0.29), or seed set per flower (OP = 0.55 ± 0.09, HP = 0.62 ± 0.06; $t_{df=24}$ = 0.202, P= 0.58).

Relation between exposure time and reproductive success

Of the 120 plants that were tagged at the beginning of the experiment, 65 plants set fruit with at least one mature seed; 12 plants could not be recovered at the end of the flowering season. Fruit set was reduced by 27-89% in flowers with experimentally shortened exposure times. Both fruit set (Fig. 2a) and seed set per flower (seed:ovule ratio, Fig. 2c) increased significantly with exposure time to pollinators (Fig. 2c), however seed set per fruit was unrelated to exposure time (Fig. 2b). There was a significant effect of exposure time (quasibinomial GLM: t = 2.80, P = 0.006) and the number of available pollinators (t = 2.87, P = 0.005) on seed set per flower, but no significant effect of plant height (t = 0.60, P = 0.55) or flower density (t = 0.79, P = 0.43).

DISCUSSION

Extended floral longevity in *Trillium grandiflorum* may be an adaptation to uncertain pollination environments characterized by daily variation in temperature and pollinator availability. Flowers with longer exposure times had a greater opportunity to be visited by pollinators and higher reproductive success than flowers with experimentally shortened exposure times. Individuals with flower life spans shorter than 10 days suffered significantly lower fertility, likely because of the reduced probability of visitation by pollinators. Extended floral longevity in *T. grandiflorum* thus increases the chances of a plant obtaining a pollinator visit, especially when there are few pollinators available in large populations of many flowering individuals. Under these circumstances, pollination is likely to be a largely stochastic process with many plants receiving no visitation in some flowering seasons. We characterize this process as 'sit-and-wait pollination', similar to the sit-and-wait model of animal foraging that occurs in environments characterized by uncertain rewards (Schoener 1971; Perry & Pianka 1994).

A patchy and uncertain pollinator environment can affect two different aspects of female reproductive success – percent fruit set and the number of seeds produced per fruit. Although these aspects of female fertility are often assumed to be roughly correlated (e.g. populations with high fruit set produce more seeds per fruit), the processes involved in fruit and seed set can be quite different. For example, in *T. grandiflorum* plants with longer exposure times to pollinators had higher fruit set than those with experimentally-shortened exposure times. However, there was no effect of exposure time on seed set per fruit. Thus, reproductive success in this species appears to be determined not by the total number of visits a flower receives, but rather whether individuals are visited by a single pollinator or not. This can occur when there is a low abundance of pollinators

but a large number of flowers available for pollination. Furthermore, the large body size of *Bombus* queens, the primary pollinator at our site, likely facilitates the deposition of substantial numbers of pollen grains to the exposed stigmas of *T. grandiflorum* during a single visit resulting in maximal seed set.

Experimental evidence supports the idea that pollination in *T. grandiflorum* is an "all or nothing" phenomenon. Studies using genetic markers have demonstrated that most flowers of *T. grandiflorum* are pollinated by a single male parent (Broyles et al. 1997; Kalisz et al. 1999), a result consistent with very low visitation rates. Moreover, controlled pollination studies using different number of pollen donors indicate that there is no fertility benefit to receiving multiple pollinator visits (Griffin & Barrett 2002). Theoretical models of optimal floral longevity assume that female reproductive success is gradually accrued throughout the flowering season as a result of multiple visits to individual flowers (Ashman & Schoen 1994, 1996; Schoen & Ashman 1995). However, our results instead suggest that for systems with low densities of pollinators, stochastic pollination is more likely to characterize the pollination process and species may have evolved traits that maximize fitness from single pollinator visits (Harder & Wilson 1992; Burd 1995).

Despite the adaptive advantages to increased flower longevity in a stochastic pollination environment, why do flowers of *T. grandiflorum* open early during unfavourable pollinator conditions and not instead open later in the spring during more favourable conditions when pollinators are more likely to be abundant? Local weather conditions and the temporal dynamics of pollinator densities can vary from year to year (e.g. Schemske et al. 1978; Barrett & Helenurm 1987; Goodwillie 2001) and long floral life spans may serve as a bet-hedging strategy to reduce the risk of reproductive failure. Daily temperatures in 2005 at our study population were generally lower than the 19-year (1987-2005) average. This variation in the spring environment may explain why the perennial *T. grandiflorum* continues to flower early. While some years have poor early spring conditions for pollination, warm temperatures and early pollinator emergence in the other years can provide high reproductive success (e.g. bet-hedging, Schemske et al. 1978). Studies across multiple seasons are required to investigate the hypothesis that variation in the early spring environment affects reproductive success. Such annual environmental variation in pollination conditions may explain why studies of pollen limitation at our site have given contrasting results; we found no evidence for pollen limitation in 2005, however pollen limitation has been previously reported in this population in both 1998 (Wright & Barrett 1999) and 1999 (Griffin & Barrett 2002). Sit-and-wait pollination through extended floral longevity may be an adaptation to stochastic pollination environments that are common to early spring-blooming species.

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