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Plasticity of floral longevity and floral display in the self-compatible biennial *Sabatia angularis* (Gentianaceae): untangling the role of multiple components of pollination

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• **Background and Aims** Plasticity of floral traits in response to pollination can enable plants to maximize opportunities for pollen import and export under poor pollination conditions, while minimizing costs under favourable ones. Both floral longevity and display are key traits influencing pollination. While pollination-induced flower wilting is widely documented, we lack an understanding of the multifactorial complexity of this response, including the influence of other pollination components, costs of extended longevity and subsequent impacts on floral display.
• **Methods** Plasticity of floral longevity was experimentally evaluated in *Sabatia angularis* in response to multiple pollination factors: pollen addition, removal, and source (self, single-donor outcross, multiple-donor outcross) and timing of pollination. Effects of pollen quantity were further evaluated by exploiting variation in autonomous self-pollen deposition. Delayed pollination costs were tested comparing seed set from early versus late pollinations. Finally, I compared floral display metrics (peak floral display, time to peak flower, flowering duration, mean flowering rate) between experimentally pollinated and control plants.
• **Key Results** Floral longevity was highly plastic in response to pollen addition and its timing, and the response was dose-dependent but insensitive to pollen source. Pollen removal tended to extend floral longevity, but only insofar as it precluded pollination-induced wilting via autonomous self-pollination. Under delayed pollination, the wilting response was faster and no cost was detected. Pollination further led to reduced peak floral displays and condensed flowering periods.
• **Conclusions** Floral longevity and display plasticity could optimize fitness in *S. angularis*, a species prone to pollen limitation and high inbreeding depression. Under pollinator scarcity, extended floral longevities offer greater opportunities for pollen receipt and export at no cost to seed set, reproductive assurance via autonomous self-pollination and larger, more attractive floral displays. Under high pollinator availability, shortened longevities lead to smaller displays that should lower the risk of geitonogamy.

Key words: Autonomous selfing, corolla wilting, delayed pollination, floral display, floral longevity, floral lifespan, plasticity, pollen limitation, pollinator scarcity, pollination, *Sabatia angularis*.

INTRODUCTION

Pollen limitation of fruit and seed production due to pollinator scarcity is pervasive across plant populations (Burd, 1994; Ashman *et al*., 2004; Knight *et al*., 2005). Accordingly, we expect strong selection for traits that promote pollination success under persistent pollen limitation (Eckert *et al*., 2010; Bartkowska and Johnston, 2015). However, accumulating evidence reveals that a population's or even an individual's pollination environment is not constant, but rather can vary across and even within reproductive seasons (e.g. Schemske and Horvitz, 1989; Ramsey, 1995; Kalisz and Vogler, 2003; Knight *et al*., 2005; Vanhoenacker *et al*., 2006). Variability in pollination services should therefore place additional selective pressure favouring plasticity in traits that influence pollinator attraction, pollen deposition and pollen export but which may otherwise be costly when pollination conditions are favourable.

Floral longevity, the length of time that flowers remain open and functional, is one trait known to have important consequences for pollination success (Primack, 1985). Longer floral lifespan can ensure sufficient pollen receipt, increase donor diversity and promote pollen export (Ashman and Schoen, 1994; Rathcke, 2003; Marshall *et al*., 2010), but at a price. Flowers serve as carbon, nutrient and water sinks during their development and throughout their lifespan (Nobel, 1977; Bazzaz *et al*., 1979; Southwick, 1984; Ashman and Schoen, 1997; Galen, 2000). Thus, the balance between daily floral fitness accrual rates (i.e. the rate at which pollen is deposited and removed) and daily floral maintenance costs are predicted to shape a species' optimal floral longevity (Ashman and Schoen, 1994; Schoen and Ashman, 1995). Consistent with theory, mean floral longevity varies markedly among plant taxa (Primack, 1985; Ashman and Schoen, 1994), and a large proportion of this variation is explained by pollination context, with greater mean floral lifespans for species under high pollen limitation (Ashman and Schoen, 1996; Rathcke, 2003).

Yet while previous models and empirical data have emphasized the heritability of mean floral longevity across species,

floral longevity can also be plastic. In particular, pollination-induced wilting responses are documented across a wide variety of taxa (e.g. Gori, 1983; Van Doorn, 1997). Plasticity of floral longevity in response to pollination could be adaptive for plants experiencing variable pollination conditions insofar as it enables further optimization of fitness, truncating longevity when fitness accrues more rapidly than on average and/or extending floral longevity in suboptimal pollination conditions where fitness accrual rates are comparatively low. An underlying assumption of this argument is that truncation of floral lifespan in response to early pollination enables plants to save costs that would otherwise be paid to maintain the flower, enabling greater investment in seeds compared to flowers experiencing delayed pollination. In contrast to the numerous cases documenting the phenomenon of pollination-induced wilting, critical tests of the fitness consequences of alterations in floral longevity associated with early versus late pollination are few (Webb and Littleton, 1987; Ashman and Schoen, 1997; Castro *et al*., 2008).

Beyond simple presence or absence of pollen, other components of pollination may influence floral longevity. These include the amount and source of pollen (self vs. outcross, few vs. many donors), the timing of pollination and pollen removal (e.g. Richardson and Stephenson, 1989; Proctor and Harder, 1995; Ishii and Sakai, 2000; Clark and Husband, 2007). Consideration of these components highlights the potential complexity of the proximate factors underlying floral longevity and exposes possible risks of a general, indiscriminant plastic response to pollen receipt. For example, pollination-induced wilting would be premature and maladaptive if it occurs in response to insufficient pollen deposition and/or before male fitness (pollen export) is fully realized (Ishii and Sakai, 2000). When in response to self-pollen receipt, pollination-induced wilting could also be maladaptive by exacerbating seed (Herlihy and Eckert, 2002) and pollen discounting (Weber and Goodwillie, 2007, 2013). Moreover, wilting in response to pollen loads with limited donor diversity could preclude opportunities for pollen competition and mate choice (Marshall *et al*., 2010). Plasticity of floral longevity that is fine-tuned to these multiple aspects of pollination would avoid these risks and probably increase fitness. Several passive or active (*sensu* Van Kleunen and Fischer, 2005) mechanisms could modulate such fine-tuned responses, including dose- and time-dependent production of the hormones that signal wilting during pollen–pistil interactions and/or ovule fertilization (Stead, 1985, 1992; Hill *et al*., 1987), slower rates of pollen germination and/or tube growth under self or single-donor pollen loads (Weller and Ornduff, 1977; Marshall, 1988; Aizen *et al*., 1990; Snow and Spira, 1991), or genotype-specific pollen–pistil interactions (Preuss, 1994; Stephenson *et al*., 1997; Lokesha and Vasudeva, 2001). Importantly, because ovule viability and the ability to discriminate among pollen types can vary with flower age (Marshall *et al*., 2010), timing of pollination may further influence the plasticity of floral longevity in response to these factors. Studies evaluating how all of these potential proximate factors may act simultaneously, and even interact, to shape floral longevity are crucial in order to fill a critical gap in our understanding of the complexity of the floral wilting response and its consequences.

The impact of plasticity in floral longevity in response to pollination is expected to extend beyond the scale of the individual flower to influence floral display (Harder and Johnson, 2005). Floral display is considered one of the most important traits influencing pollinator behaviour and hence plant fitness (Devaux *et al*., 2014). Large floral displays promote outcross pollen receipt because they are more attractive to pollinators (e.g. Mitchell, 1994; Harder and Barrett, 1995; Snow *et al*., 1996; Karron and Mitchell, 2012). On the other hand, they also encourage pollinators to visit many flowers within a plant, potentially leading to increased self-pollination and decreased pollen export (e.g. Klinkhamer and de Jong, 1993; Harder and Barrett, 1995; Snow *et al*., 1996; Mitchell *et al*., 2004; Lau *et al*., 2008; Karron and Mitchell, 2012). Harder and Johnson (2005) highlighted the perspective that pollination-induced floral wilting could 'serve the entire plant' by enabling plastic adjustments of floral display size in response to pollination conditions in a way that can resolve this dilemma and increase fitness. When pollination rates are low, plastically extended floral lifespans would lead to larger, more attractive displays, whereas under favourable pollination conditions, shortened floral lifespans would lead to smaller displays that should reduce the risks of pollinator-mediated selfing among flowers (geitonogamy). Plasticity of floral lifespans could further impact flowering duration, another component of floral display, in a way that could also be adaptive. Longer lifespans under pollen limitation could translate into longer total flowering duration, extending opportunities to accrue fitness, while shorter floral lifespans under high pollination rates and subsequent compression of flowering duration may enable avoidance of resource depletion or higher seed predation rates later in the season (Elzinga *et al*., 2007). Importantly, because floral display and flowering duration are aggregate traits, dependent not only on the longevity of individual flowers but also the rate at which flowers open, there may be more than one mechanism by which they could respond to pollination (Harder and Johnson, 2005). Despite the clear links between plasticity in floral longevity and display, the influence of pollination conditions on the latter has scarcely been examined (Karrenberg and Jensen, 2000; Harder and Johnson, 2005).

In this study, I present a set of comprehensive and complementary experiments investigating the effect of multiple components of pollination on floral longevity and floral display in *Sabatia angularis* (Gentianaceae), a self-compatible biennial prone to pollen limitation (Dudash, 1993; Spigler and Chang, 2009). Specifically, I asked the following questions: (1) What are the effects of pollination and pollen removal on floral longevity? (2) Is the plastic response of floral longevity to pollen deposition dose-dependent? (3) To what extent does pollen source (self, single-donor outcross and multiple-donor outcross), timing of pollination and their interaction influence floral longevity? (4) What is the fitness consequence of early vs. delayed pollination in terms of seed production, and does it depend on source of pollen received? Finally, (5) how does pollination influence floral display parameters? That is, are effects on floral display and flowering duration mediated solely through changes in floral longevity, or does pollination also influence the rate of flowering? Together, these studies shed light on the complexity of factors influencing plasticity of key reproductive traits with implications for fitness.

MATERIALS AND METHODS

Study species

Sabatia angularis occurs in a variety of habitats such as prairies, marshes, rocky outcrops, old fields and roadsides throughout eastern USA and south-eastern Canada. Seeds germinate in spring, and seedlings develop into rosettes that overwinter until the following year. From July to August, plants produce displays of showy, nectarless, pink flowers that are visited by a generalist suite of pollinators, including leaf-cutter bees (Megachilidae), sweat bees (Halictidae), andrenid bees (Andrenidae), small carpenter bees (Anthophoridae) and hover flies (Syrphidae) (my pers. obs.). Individual flowers are protandrous; during male phase, the two stigma lobes remain wrapped around each other. Female phase typically begins by the end of the second day flowers are open, at which point the stigma lobes unwrap into a 'Y' shape. Observations from a previous field study found that by day four of anthesis approx. 90 % of the pollen is removed from flowers and most are pollinated (Dudash, 1991). Pollinated flowers develop into dry dehiscent capsules often containing hundreds to over a thousand seeds (Dudash, 1991; Spigler and Chang, 2008).

Sabatia angularis is mixed mating to highly outcrossing (mean $t_m = 78 \pm 0{\cdot}12$ % SD, $n = 8$ populations; Spigler *et al*., 2010), but primary selfing rates have been estimated to be as high as 49 % (R. B. Spigler *et al*., unpubl. res.). Selfing probably occurs through a mixture of autonomous selfing within a flower when male and female phases overlap and movement of self pollen among flowers within a plant by pollinators. The latter is particularly likely given that an individual can have tens to upwards of 100 flowers open at a given time. Previous studies reveal high levels of inbreeding depression across the life cycle ($\delta > 0{\cdot}5$; Dudash, 1990; R. B. Spigler *et al*., unpubl. res.). However, there is no early acting inbreeding depression (Dudash, 1990; R. B. Spigler *et al*., unpubl. res.).

Floral longevity experiments

Plants for the floral longevity experiments originated from open-pollinated seed collected from 50 plants (families) in a serpentine grassland population in south-eastern Pennsylvania ('UB5', 39°54·810′N, 75°42·711′W). Seeds were planted in a randomized block design, cold stratified and germinated in Temple University's Plant Growth Facility. Approximately 3 months later I randomly selected one rosette per family, transplanted each into a 5-inch square pot filled with a 3 : 1 Fafard 3b (Sungro Horticulture, Agawam, MA, USA)/Turface (PROFILE Products LLC, Buffalo Grove, IL, USA) mix, and raised them to flower in a pollinator-free growth chamber, where they were watered and fertilized regularly throughout the flowering period. A final total of 41 plants were included in the experiment.

To first test for the effects of pollen deposition and pollen removal on floral longevity, on all plants I assigned flower buds to one of the following treatments: control, pollen deposited, anthers removed (emasculated), or anthers removed plus pollen deposited (Table 1A). Control flowers had their anthers left intact and were not given outcross pollen. Pollinations were performed using outcross pollen from a single donor applied on the first day after onset of female phase. Entire anthers were removed to simulate pollen removal, an approach also employed by Ishii and Sakai (2000). In this way, the anther removal treatment represents prevention of autonomous self-pollen deposition in addition to completion of male function. I suspected that variation in autonomous selfing ability might inflate variation in measurements on control flowers; therefore, I included two replicates of this treatment per plant and one replicate for all other treatments.

To test the effects of pollen source, timing of pollination, and their interaction, I employed the following pollination treatment levels: self ('S') pollen applied on day 1 of female phase, outcross pollen from a single donor ('O_1') applied on day 1 [note this is the same as the 'pollen deposited' treatment above], outcross pollen from five donors ('O_5') applied on day 1, and each of S, O_1 and O_5 on day 5 of female phase (Table 1B). Because most flowers recorded in the wild are female at end of the second day a flower is open, the 'day 1' and 'day 5' female phase treatments used here were chosen to approximate the third and seventh day flowers are open, respectively. Compared to the observation that flowers are pollinated by day 4 of anthesis in the field (Dudash, 1991) and assuming floral longevities in the field closely align with optimal floral longevity (Ashman and Schoen, 1994) these timings should correspond to pollination before and after optimal longevity, respectively.

For both experiments, pollinations were conducted by collecting the appropriate dehisced anthers in a 1·5-μL microtube and painting pollen on stigmas with a fine paintbrush until they were visibly covered with the yellow pollen. This represents saturation of female function (mean 2721 grains ± 990 s.d., $n = 10$ stigmas). Outcross donor pollen for a given flower was chosen haphazardly from a stock of donor plants originating from the same population reared alongside the experimental plants. For O_5 treatments, we collected an equivalent number of anthers from each of five donors and mixed them in the microtube. Self-pollen was collected from non-treatment flowers on the same plant.

Flowers were checked daily throughout the duration of the experiment to determine: date of flower opening; date female phase began; date of pollen deposition when applicable (corresponding to either day 1 or day 5 of female phase); date of stigma wilting; and date of corolla wilting. Stigmas were considered wilted when at least the tips of both stigma lobes turned brown. Corolla wilting was acknowledged when at least three of the five petals had curled. From these data, I determined two metrics of floral longevity: (1) stigma longevity, the number of days between flower opening and stigma wilting; and (2) corolla longevity, the number of days between opening and corolla wilting. I included both metrics because they are complementary, but not necessarily identical; whereas stigma longevity should be tied intimately to female function only, corolla traits are often considered to serve male function (e.g. Bell, 1985). Consequently, these two may have divergent plastic responses, particularly to pollination and pollen removal. Finally, I also determined 'wilting response time' for stigmas and corollas as the amount of time it took each to wilt after pollination. This measure provides complementary information to floral longevity by describing the *rate* of the plastic response to pollination, which may vary with flower age at the time of pollination (e.g. Webb and Littleton, 1987).

TABLE 1. *Treatments employed in factorial floral longevity experiments and their abbreviations*

A. Testing the effects of pollen deposition and pollen (anther) removal on floral longevity

Pollen (anther) removal	Pollen deposition	
	None	Pollen deposited
Anthers intact	C	+P*
Anthers removed	−A	−A+P

B. Testing the effects of pollen source and timing of pollination on floral longevity

Pollen source	Timing (day of female phase)	
	Day 1	Day 5
Self	S1	S5
Outcross: single donor	$O_1$1	$O_1$5
Outcross: multiple (5) donors	$O_5$1	$O_5$5

*Pollen deposition treatment represents outcross pollen from a single donor applied on day 1 of female phase.

To evaluate whether the plastic response of floral longevity to pollen deposition is dose-dependent, I took advantage of natural variation in autonomous self-pollen deposition and collected stigmas from intact, control flowers (one replicate per plant) once flowering had ceased and fruits developed. I softened stigmas in 8 M NaOH and visualized the naturally autofluorescent pollen grains on an epifluorescence microscope at 4× magnification. I then enumerated the number of pollen grains on one of the two stigma lobes, selected at random.

Finally, to evaluate the potential cost of delayed pollination for seed production, I collected and weighed fruits resulting from early (day 1) and late (day 5) pollinations of all pollen source types (S, O_1, O_5) (Table 1B). Previous work demonstrated that fruit mass is an excellent surrogate for seed set (Spigler and Chang, 2008).

All data analyses were generated using SAS software v. 9.3 (SAS Institute Inc., Cary, NC, USA). I used general linear mixed models (proc mixed) to evaluate comparisons among experimental treatments. First, I tested for the effects of pollen deposition and anther removal on floral longevity (question 1). Data for this analysis came from treatments listed in Table 1A. Predictor variables in the model were pollen deposition, anther removal and their interaction; response variables were stigma and corolla longevity. Second, I tested whether pollen source (S, O_1, O_5), timing of pollination (day 1 vs. 5) and their interaction influence stigma and corolla longevity and wilting response times (question 3). Data for this analysis came from treatments listed in Table 1B. To evaluate potential costs of delayed pollination for seed set (question 4), I tested the effects of pollen source, timing of pollination and their interaction on fruit mass. In all models, plant identity was included as a random effect. To account for and to evaluate possible trade-offs between individual floral longevity and total flower number (Schoen and Ashman, 1995), I initially included total flower number per plant as a covariate in all models; I retained it in the model only where significant ($P < 0{\cdot}05$). When an interaction was significant, I report *t*-tests and *P*-values from the solution table for the original model. Otherwise, *F*-tests based on Type III SS are presented. In the case of significant interactions, I further proceeded by testing whether the simple effects for each factor are significant using the SLICE statement. Evaluation of box plots revealed unequal variances between some treatment levels. Therefore, I accounted and tested for heterogeneity in the covariance structure using the 'group' option for each factor where applicable in a repeated statement in proc mixed (Littell *et al*., 1996). The significance of heterogeneity of variances between the pollination treatments was evaluated comparing Akaike information criterion (AIC) scores of models with and without each grouping factor using $\Delta AIC > 2$ criterion. I used Satterthwaite's approximation to determine the degrees of freedom in all mixed models. Finally, I used general linear models (proc glm) to evaluate the relationship between floral longevity (stigma and corolla) and the number of pollen grains per stigma (question 2). Because this relationship was non-linear, I log-transformed pollen grains per stigma. Residuals of all models were inspected to evaluate their conformity to model assumptions.

Floral display experiment

Concurrently with the floral longevity study, I conducted a complementary experiment to test the impact of pollination on metrics of floral display. Plants for the floral display experiment came from two sources: the same collection of UB5 seeds as in the longevity experiment and, to increase the sample size, plants reared from seed collected in a second population ('GFR', 40°15′16·39″N, 75°30′33·78″W). These were grown alongside plants included in the longevity experiment and were randomly assigned to either a pollination treatment or control group (evenly divided between populations). A total of 42 plants were included ($n = 21$ per treatment). Three times per week, all new female-phase flowers on treatment plants were pollinated with supplemental outcross pollen collected from donor plants not included in the experiment (one donor per flower). Donor pollen was always matched to the same population as the focal treatment plant. This was repeated throughout the duration of the study until all flowers per plant were pollinated (plants varied in flower number from 33 to 247). I did not pollinate control plants; however, they retained any ability to self-pollinate autonomously. I checked all plants three times per week over the duration of the experiment and recorded: date first flower opened, daily open flower number, date last flower bud opened and date last flower wilted. Flower wilting was defined with respect to the corolla as described above. I also counted the total number of flowers produced per plant. From these data, I determined for each plant: peak (maximum) floral display; time to peak display (number of days); total flowering duration as number of days from the first flower opened until the last flower wilted; and mean flowering rate as: [adjusted total flower number]/[number of days until the last flower bud opened]. Adjusted total flower number accounts for the fact that plants had variable numbers of flowers open on day 1, prior to application of any treatments, and was calculated as: [total flower number] – [number of flowers open on day 1].

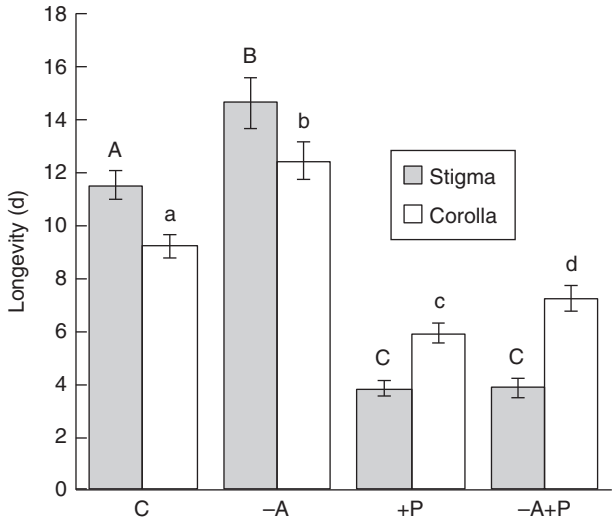

FIG. 1. Floral longevity measured as time (d) until wilt of stigma (grey bars) and corolla (white bars), under different pollination conditions. Treatments are represented as: C = intact control, −A = anthers removed, +P = pollen deposited (outcross pollen from a single donor on first day after onset of female phase), −A+P = anthers removed plus pollen deposited. Least squares means ± s.e. are shown. Different letters above bars indicate significant differences between treatments based on *post hoc* tests; capital letters are used for comparisons of stigma wilting and lower case letters for corolla wilting.

I used general linear mixed models (proc mixed) to evaluate the effect of pollination on metrics of floral display (question 5). Response variables were peak floral display, time to peak display, total flowering duration and mean flowering rate. Fixed effects in each model were pollination treatment and population identity. The interaction between pollination treatment and population was initially evaluated, but never significant ($P > 0{\cdot}05$). Because I was interested in main effects, I removed these non-significant interactions from all analyses. Total flower number was included as a covariate in analyses of peak floral display, time to peak display and total flowering duration and retained only where it was significant ($P < 0{\cdot}05$). Heterogeneity of variance between pollination groups and populations was evaluated and incorporated and Satterthwaite's approximation was used to determine degrees of freedom, where appropriate, as described above. Residuals of all models were also inspected to evaluate their conformity to model assumptions.

RESULTS

Floral longevity

Flowers that were hand-pollinated had significantly shorter lifespans than unpollinated flowers, in terms of both stigma and corolla longevity (Fig. 1, Table 2). On average, stigmas of flowers that did not receive a pollen addition treatment ('C' and '–A') remained turgid and yellow for 13·1 d (±0·57 s.e.), whereas stigmas of pollinated flowers ('+P' and '–A+P') lasted only 3·8 d (±0·26 s.e.), representing a 71 % reduction in stigma longevity in response to pollination. Corollas on flowers that did not receive a pollen addition treatment lasted an average of 10·8 d (±0·47 s.e.) compared to only 6·6 d (±0·35 s.e.) on pollinated flowers, a 39 % decrease in longevity. Comparison of the maximum longevity seen between pollen addition levels further illustrates the magnitude of this difference: stigmas and corollas on unpollinated flowers were found to last as long as 26 and 20 d, respectively, but only as long as 9 and 7 d, respectively, under the pollen addition treatment. Anther removal did not influence stigma longevity on average across pollen addition treatments, but the interaction was significant, revealing that anther removal only influenced stigma longevity on flowers that did not receive the pollen (Fig. 1, Table 2, Supplementary Data Table S1). For those flowers, anther removal resulted in *greater* longevity. Anther removal increased corolla longevity on average, and the significant interaction with pollen addition revealed that the magnitude of this effect is dampened when flowers are pollinated (Fig. 1, Table 2, Table S1). Stigmas invariably wilted prior to corollas on flowers that were hand pollinated, but, unexpectedly, the reverse occurred in flowers left unpollinated: stigmas remained turgid long after the corolla wilted (Fig. 1: compare heights of grey and white bars for treatments C and –A vs. +P and –A+P).

TABLE 2. *Model results for effects of pollination, anther removal and their interaction on floral longevity*

Effect	Stigma longevity			Corolla longevity		
	d.f.	*t**	*P*	d.f.	*t**	*P*
Pollination	105	10·46	<0·0001	109	6·62	<0·0001
Anther removal	30·4	−0·09	0·93	44·1	−2·7	0·01
Pollination × Anther removal	107	−2·58	0·01	113	−2·05	0·04

*Because of significant interaction effects, *t*-test and associated *P* values are presented instead of *F*-tests.

Models accounting for heterogeneity in variance between pollination treatments were strongly favoured over those that did not (stigma longevity ΔAIC = 46·3, corolla longevity ΔAIC = 13·2) and revealed that variation in floral longevity was significantly reduced under pollination. For stigma longevity, this difference was an order of magnitude, with a variance estimate of 2·5 d for pollinated flowers compared to 21·6 d for flowers that were not pollinated. For corolla longevity, variation in time until corolla wilt was only 3 d for pollinated flowers, but 10·9 d for those left unpollinated.

Based on natural variation in pollen deposition among intact control flowers, floral longevity decreased non-linearly with greater pollen grain deposition. Specifically, both stigma ($F = 5{\cdot}71$, $P = 0{\cdot}02$, $R^2 = 0{\cdot}13$, $n = 39$) and corolla ($F = 7{\cdot}24$, $P = 0{\cdot}01$, $R^2 = 0{\cdot}16$, $n = 39$) longevity decreased with the logarithm of the number of grains deposited (Fig. 2).

Comparisons of floral longevity under the factorial combination of pollen source and timing of pollination revealed that floral longevity was plastic only in response to timing (Table 3, Fig. 3). Pollen source had no influence nor was the interaction between source and timing significant. Further comparisons of the rate of the wilting response of both stigmas and corollas revealed that the response was significantly more rapid for flowers pollinated later (day 5) compared to those pollinated earlier (day 1) (Table 3). In fact, day 5 flower stigmas and corollas wilted in almost half the time post-pollination as day 1 flowers (Fig. 4). There was greater variability in stigma longevity

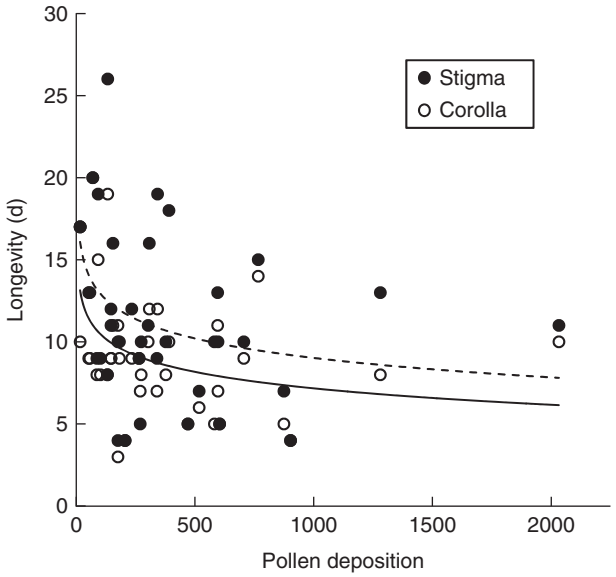

FIG. 2. Floral longevity decreases as a function of pollen deposition. Pollen deposition was measured as the number of pollen grains per stigma deposited autonomously on intact control flowers. Trend lines illustrate that both stigma (dotted line) and corolla (solid line) longevity decrease with the logarithm of pollen deposition.

among those pollinated on day 1 than day 5 (2·9 vs. 0·9 d, respectively) (ΔAIC = 13·9). Similar differences occurred for variation in stigma wilting response time between day 1 and day 5 flowers (2·0 vs. 0·40 d, respectively) (ΔAIC = 55·7).

Despite significant differences in floral longevity between flowers pollinated on day 1 and day 5, there was no difference in resultant fruit mass ($F_{1,166} = 0{\cdot}93$, $P = 0{\cdot}33$). Pollen source similarly had no impact on mean fruit mass ($F_{2,168} = 1{\cdot}83$, $P = 0{\cdot}16$) nor did its interaction with pollination timing ($F_{1,168} = 0{\cdot}81$, $P = 0{\cdot}45$). Variance in fruit mass was greater among flowers pollinated on day 1 than on day 5 ($2{\cdot}4 \times 10^{-4}$ vs. $1{\cdot}0 \times 10^{-4}$ g; ΔAIC = 13·6), similar to results for stigma longevity and wilting response time. There was a significant trade-off between total flower number seed set ($F_{1,35{\cdot}9} = 25{\cdot}16$, $P < 0{\cdot}0001$).

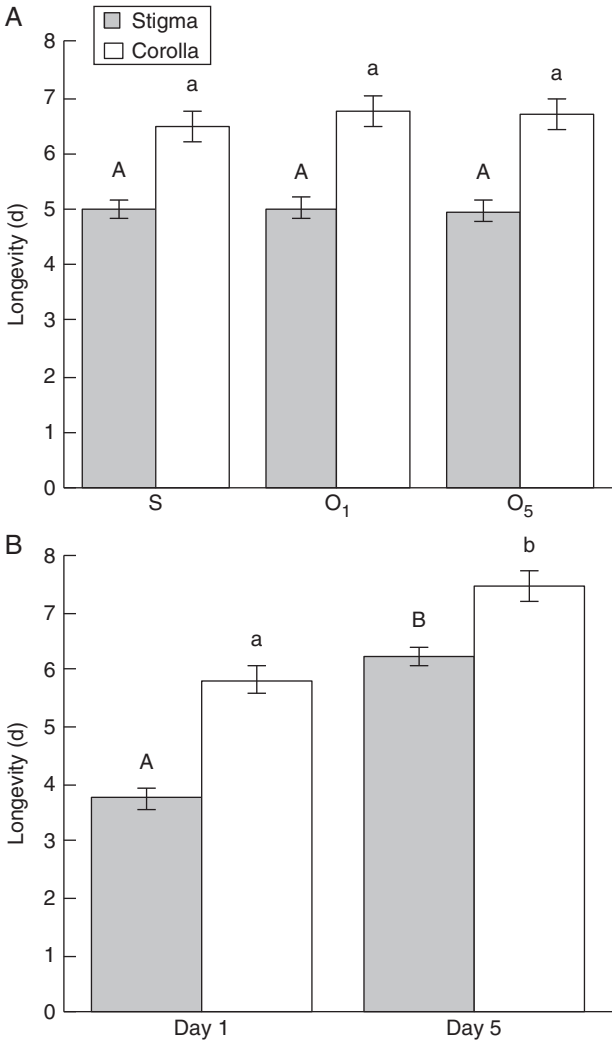

FIG. 3. Floral longevity, measured as time (d) until wilt of stigmas (grey bars) and corollas (white bars), under different pollination conditions. (A) Average effect of self (S), outcross pollen from a single donor (O_1) and outcross pollen from five donors (O_5). (B) Average effect of timing of pollination. Least squares means ± s.e. are shown. Different letters above bars indicate significant differences between treatments; capital letters are used for comparisons of stigma wilting and lower case letters for corolla wilting.

Floral display

Peak floral display ranged from 16 to 151 flowers per plant across the experiment. Pollinated plants had significantly smaller floral displays at their peak than control plants, accounting for individual variation in total flower number (Table 4, Fig. 5). This decrease was substantial, representing a 21·6 % decrease in the number of flowers open at peak flower. Both types of plants reached peak flower at approximately the same time (Table 4, Fig. 4). Flowering duration was significantly shorter for pollinated plants (Table 4, Fig. 5), representing a 20·4 % decrease in the number of days that plants flowered as compared to control plants. Mean flowering rate, however, was remarkably similar between treatments, with flower buds opening at a rate of 5·3 buds d^{-1} averaged over the duration of flowering (Table 4, Fig. 5). Only populations differed in their mean flowering rate (Table 4), with UB5 opening approx. 2·4 more flowers d^{-1} than population GFR.

DISCUSSION

Plasticity in floral longevity considering multiple dimensions of pollination

The results of this study demonstrate that *S. angularis* is highly plastic in response to multiple pollination factors, specifically pollen deposition, amount and timing. The reduction in floral longevity in response to hand pollination was striking, and in conjunction with prior demonstrations of pollination-induced wilting (e.g. Gori, 1983; Richardson and Stephenson, 1989; Proctor and Harder, 1995; Ishii and Sakai, 2000; Evanhoe and Galloway, 2002; Stpiczyńska, 2003; and reviewed by Van

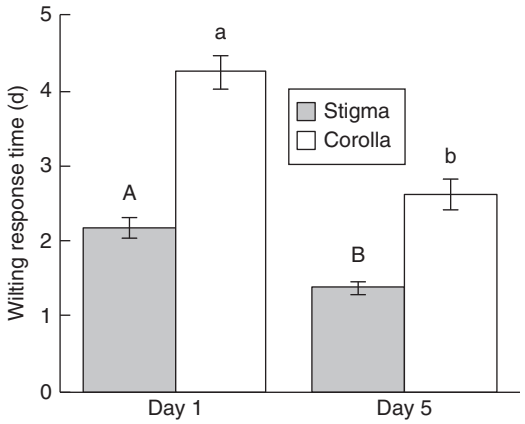

FIG. 4. Wilting response time, measured as number of days from pollination until wilting of stigmas (grey bars) and corollas (white bars), of flowers pollinated on day 1 and 5 after onset of female phase. Least squares means (averaging over pollen sources) ± s.e. are shown. Different letters above bars indicate significant differences between treatments; capital letters are used for comparisons of stigma wilting and lower case letters for corolla wilting.

Doorn, 1997) highlights the ubiquity of this response. Importantly, the data also revealed that the response is dose-dependent. Below approx. 200 grains, longevity is equivalent to emasculated flowers; above this amount, longevity continues to decline with increasing pollen receipt, with a minimum longevity approximating that seen under full hand pollination (~6 d). Dose-dependency has been found in a few other herbaceous species as well (Ishii and Sakai, 2000; Lokesha and Vasudeva, 2001; Clark and Husband, 2007) and is expected to maximize opportunities for sufficient pollination. These results at the level of individual flowers fit well with theory predicting optimal floral longevity for taxa based on fitness accrual rates (Ashman and Schoen, 1994; Schoen and Ashman, 1995). Given variable rates of pollen deposition across *S. angularis* populations, ranging from ~100 to more than 600 grains on average (Dudash, 1991; Spigler and Chang, 2008), I expect similar differences in longevity in the field. Indeed, mean longevity can even change within populations across years in relation to pollination context (Castro *et al.*, 2008). These lines of evidence suggest that general theory on floral longevity and the expected relationship between floral longevity and pollen limitation seen across species (Ashman and Schoen, 1996) should extend to individual- and population-level variation within species.

In contrast, the pollination-induced wilting response in *S. angularis* was insensitive to the source of pollen deposited. Because this response is triggered by hormone signalling initiated during pollen-pistil interactions and/or ovule fertilization (reviewed by O'Neill, 1997), differential sensitivity of floral longevity may only be possible where pollen source affects these processes. Such differences were found to lead to greater longevity under self compared to outcross pollination in two cases, including a self-compatible species (Richardson and Stephenson, 1989; Lokesha and Vasudeva, 2001). In *S. angularis*, equivalent seed fertilization rates among self, single donor outcross and multiple donor outcross pollen suggests the species lacks such a mechanism to discriminate among these pollen sources. For self vs. outcross pollen, the fitness consequences of the inability to discriminate are likely to depend on the mode of selfing. Lloyd and Schoen (1992) and Lloyd (1992) outlined how different modes of selfing could lead to pollen and seed discounting, but these models did not account for plasticity in floral lifespan. If pollinators mediate selfing, extended longevity under self-pollination should not increase outcross pollen receipt or export. In this case, the cost of selfing via its impacts on floral longevity should be no different from the cost of selfing itself. Early autonomous selfing, on the other hand, could exacerbate both seed and ovule discounting by truncating floral lifespan before opportunities for outcross pollen receipt and pollen export by pollinators have passed. Autonomous selfing in *S. angularis* indeed truncated floral longevity of control flowers relative to the maximum seen in emasculated flowers, similar to patterns found in self-compatible *Leptosiphon* (Weber and Goodwillie, 2007, 2013) and *Impatiens* spp. (Sato, 2002). However, control *S. angularis* flowers nevertheless still lived nearly three and two times as long as day 1 and day 5 hand-pollinated flowers, respectively (Fig. 1), reflecting either delayed autonomous selfing or substantially lower mean pollen deposition through autonomous selfing (383 grains ± 392 s.d.) relative to hand pollinations. Either suggests that the fitness impact of selfing via its influence on floral longevity may be minimal.

Effects of male function on floral longevity have been less well examined compared to those of female function via pollination. Reports are conflicting, with some demonstrating shortened longevity in response to pollen removal (Richardson and Stephenson, 1989; Huda and Wilcock, 2012; Dart and Eckert, 2013) but others showing no effect (Ishii and Sakai, 2000; Arathi *et al.*, 2002; Abdala-Roberts *et al.*, 2007; Castro *et al.*, 2008). In *S. angularis*, pollen removal via emasculation instead extended longevity, but mainly for unpollinated flowers. The increased longevity, while unexpected, is best explained as an indirect response; removal precludes autonomous self-pollination and thus pollination-induced wilting. Consequently, pollen removal per se does not appear to directly impact longevity in *S. angularis*. One caveat is that I removed entire anthers, and the extent to which this leads to a different physiological response than pollen removal by insects remains to be tested. Nevertheless, that the cues for plasticity of floral longevity are female-centric in *S. angularis* is consistent with predictions for protandrous species, where male function precedes female function (Ashman and Schoen, 1996; Ishii and Sakai, 2000). In such species, plasticity in response to pollen removal might actually be maladaptive if it resulted in truncation of floral lifespan prior to completion of female function.

Although the proximate forces influencing plasticity of floral longevity in *S. angularis* are mediated solely by completion of female function, the ultimate evolutionary forces need not be. In particular, the greater longevity of corollas than stigmas under hand-pollination (Fig. 1) evokes the role of selection through male fitness. While this response might merely represent a non-adaptive physiological constraint, for example because it takes longer for the corolla than the stigma to receive and/or respond to hormonal signals (reviewed by O'Neill, 1997), postponed corolla wilting can provide a minimum floral longevity needed to complete male function (Ishii and Sakai, 2000). A longer time to corolla wilt when stigmas are pollinated early than late (Fig. 4) would further allow for this minimum. Indeed, Dudash (1991) reported that pollen continues to be

TABLE 3. *Model results for fixed effects of pollen source, timing of pollination and their interaction on floral longevity and wilting response*

	Stigma				Corolla			
Effect	Num d.f.	Den d.f.	*F*	*P*	Num d.f.	Den d.f.	*F*	*P*
A. Floral longevity								
Pollen source	2	167	0·02	0·98	2	184	0·89	0·41
Timing of pollination	1	168	227·82	<0·0001	1	185	79·26	<0·0001
Pollen source × Timing	2	167	0·27	0·76	2	184	0·32	0·72
B. Wilting response								
Pollen source	2	142	0·37	0·69	2	175	0·41	0·67
Timing of pollination	1	143	29·44	<0·0001	1	176	96·00	<0·0001
Pollen source × Timing	2	143	0·93	0·40	2	175	0·11	0·89

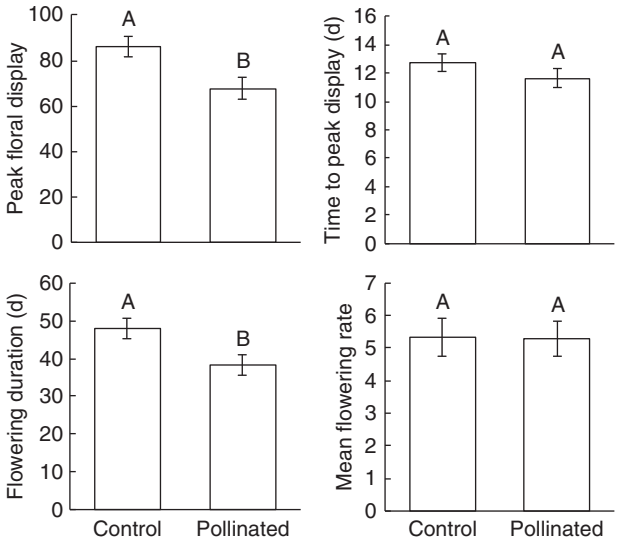

FIG. 5. Effect of pollination on floral display, time to peak flower, flowering duration and mean flowering rate. Least squares means ± s.e. are shown; different letters above bars indicate significant differences between treatments.

TABLE 4. *Model results for fixed effects of pollination and population on components of floral display*

Effect	Num d.f.	Den d.f.	*F*	*P*
A. Peak floral display				
Pollination	1	37	8·92	0·005
Population	1	37	1·93	0·17
Total flower number	1	37	59·23	<0·0001
B. Time to peak flower				
Pollination	1	39	1·66	0·21
Population	1	39	0·61	0·44
C. Total flowering duration				
Pollination	1	39	7·71	0·008
Population	1	39	3·53	0·07
D. Mean flowering rate				
Pollination	1	29·5	0	0·95
Population	1	13·3	7·18	0·02

removed from flowers days after stigmas become receptive in *S. angularis*. Wilting of stigmas prior to corollas might further serve to reduce interference with later pollen removal and export (Lloyd and Yates, 1982; Barrett, 2002) or represent the outcome of sexual conflict and manipulation by male gametophytes to secure paternity (Lankinen *et al*., 2006).

Theory on the selective forces shaping floral longevity emphasizes not only the potential benefits of increased floral longevity but also its costs. If flower maintenance requires resources, a prediction is that extended floral lifespans due to delayed pollination will result in fitness losses relative to shorter lifespans under early pollination. Findings from a few other studies support this prediction, revealing reduced seed set under delayed relative to early pollinations (Ashman and Schoen, 1997; Castro *et al*., 2008; Marques and Draper, 2012). In contrast, I found no difference in seed production as measured by total fruit mass despite significantly longer floral lifespans under delayed pollination (Fig. 2), suggesting minimal daily floral maintenance costs in *S. angularis*. Low floral costs in *S. angularis* could be attributable, in part, to the absence of nectar, which can represent a substantial expense (Southwick, 1984; Pyke, 1991). Faster wilting under delayed pollination might also minimize costs. Admittedly, my ability to detect costs may have been limited. For one, floral maintenance costs can vary with resource availability (Ashman and Schoen, 1997) and may have been alleviated in the relatively resource-rich greenhouse conditions. Second, the delayed pollination treatment may have simply been too early to detect costs given the maximum lifespan seen in this experiment. If costs are realized once *S. angularis* flowers are older, perhaps this could explain another observation: in contrast to the sequence of wilting for pollinated flowers, corollas on *unpollinated* flowers wilted *before* stigmas (Fig. 1). Earlier wilting of corollas – as early as 9 d before stigma wilting – under severe pollen limitation could present a cost savings scenario, enabling flowers to shed putatively (more) costly petals while retaining the chance to set seed. Future work will evaluate this hypothesis by testing for costs in substantially older flowers and under more realistic field conditions.

Plasticity in floral display is largely mediated by plasticity in floral longevity

The finding of low costs to delayed pollination in terms of seed set, however, begs the question as to why *S. angularis* flowers do not then always last longer, particularly if complete seed set has not been achieved. One hypothesis is that the rapid

corolla wilting response to pollination may have instead been selected via its influence on floral display as it provides a solution to the trade-offs faced in attracting pollinators (Harder and Johnson, 2005). The combined results of the floral longevity and display experiments here clearly illustrate this mechanism. Pollinated *S. angularis* plants had significantly reduced peak floral displays compared to control plants, similar to results found in only two other studies (Karrenberg and Jensen, 2000; Harder and Johnson, 2005). Moreover, pollinated plants had condensed flowering periods. Both responses could have fitness consequences for *S. angularis* plants. First, consistent with the classic dilemma, large, many-flowered *S. angularis* plants enjoy a pollen deposition and seed set advantage over plants with few flowers but are also more likely to experience a greater number of sequential pollinator visits within a plant (Dudash, 1991). Second, patterns of selection across populations in *S. angularis* reveal selection for increased flowering duration where mean population pollen receipt is low (S. L. Emel, S. J. Franks and R. B. Spigler, unpubl. res.). Given variable pollen receipt across years (R. B. Spigler, unpubl. res.) plasticity in display may allow *S. angularis* plants to enjoy the benefits of greater floral displays and extended flowering duration under high pollen limitation while avoiding or reducing risks (geitonogamy, resource depletion or predation) when pollinators are abundant.

Plasticity in display and duration could further be mediated through changes in flowering rate in response to pollination (Harder and Johnson, 2005). Although flowering rate in *S. angularis* as measured here was not affected by pollination, this metric represents an average across the entire flowering period. Other observations suggest that the picture is more complex. In particular, the finding of equivalent mean flowering rates between treatments is at odds with the finding that control plants have larger peak displays than pollinated ones but reach their peak at approximately the same time. Instead, this implies elevated rates of at least initial flower opening for control plants or reduced rates in pollinated plants. Consequently, flowering rate is unlikely to be constant across the flowering period and may in fact be influenced by pollination.

CONCLUSIONS

This paper highlights the complexity of factors influencing floral longevity and the possible fitness effects. These effects implicate not only direct selection on plasticity in floral longevity but also indirect selection via its influence on floral display, with consequences for the mating system. Whereas floral longevity and display are often considered and modelled as canalized traits moulded via selection, the results presented here combined with accumulating knowledge of the unpredictability of pollination conditions call for the extension of models predicting a fixed optimum to those considering the evolution of plasticity in longevity and display in response to variable fitness accrual rates.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: tests of simple effects for each factor considering significant interactions between pollination and pollen (anther) removal on floral longevity.

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