

Floral longevity and autonomous selfing are altered by pollination and water availability in *Collinsia heterophylla*

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• **Background and Aims** A plant investing in reproduction partitions resources between flowering and seed production. Under resource limitation, altered allocations may result in floral trait variations, leading to compromised fecundity. Floral longevity and timing of selfing are often the traits most likely to be affected. The duration of corolla retention determines whether fecundity results from outcrossing or by delayed selfing-mediated reproductive assurance. In this study, the role of pollination schedules and soil water availability on floral longevity and seed production is tested in *Collinsia heterophylla* (Plantaginaceae).

• **Methods** Using three different watering regimes and pollination schedules, effects on floral longevity and seed production were studied in this protandrous, flowering annual.

• **Key Results** The results reveal that soil water status and pollination together influence floral longevity with low soil water and hand-pollinations early in the floral lifespan reducing longevity. However, early pollinations under excess water did not extend longevity, implying that resource surplus does not lengthen the outcrossing period. The results also indicate that pollen receipt, a reliable cue for fecundity, accelerates flower drop. Early corolla abscission under drought stress could potentially exacerbate sexual conflict in this protandrous, hermaphroditic species by ensuring self-pollen paternity and enabling male control of floral longevity. While pollination schedules did not affect fecundity, water stress reduced per-capita seed numbers. Unmanipulated flowers underwent delayed autonomous selfing, producing very few seeds, suggesting that inbreeding depression may limit benefits of selfing.

• **Conclusions** In plants where herkogamy and dichogamy facilitate outcrossing, floral longevity determines reproductive success and mating system. Reduction in longevity under drought suggests a strong environmental effect that could potentially alter the preferred breeding mode in this mixed-mated species. Extrapolating the findings to unpredictable global drought cycles, it is suggested that in addition to reducing yield, water stress may influence the evolutionary trajectory of plant mating system.

Key words: Autonomous selfing, *Collinsia heterophylla*, drought stress, floral longevity, herkogamy, Plantaginaceae, reproductive success, resource allocation, sexual conflict.

INTRODUCTION

Floral longevity, defined as the duration of time a flower is open and receptive to pollination, is a crucial measure that influences plant reproductive success and can be invoked to explain the extensive variation in angiosperm reproductive strategies (Primack, 1985; Schoen and Ashman, 1995). Flowers are the reproductive organs that protect male and female structures as well as play a significant role in attracting pollinators to facilitate outcrossing (Darwin, 1876) and they are metabolically expensive as turgor in floral organs needs to be maintained throughout the floral lifespan despite sustained evapo-transpiration (Carroll *et al.*, 2001). Cost–benefit models of longevity, describing the relationship between resource trade-offs and flower maintenance, suggest that long-lived flowers are selectively favoured when their fitness accrual rates and the cost for the maternal plant to maintain them are both low and short-lived flowers are favoured when maintenance costs and fitness accrual rates are both high (Schoen and Ashman, 1995). Flowers should therefore exhibit optimal longevity that maximizes fitness accrual at minimum costs to the maternal plant, an optimum which can be expected to vary with the environment, mating system,

pollination success and plant species (Gori, 1983; Primack, 1985; Schoen and Ashman, 1995; van Doorn, 1997).

Physiological cues emanating from the completion of sexual functions induce floral senescence but the specific sex that is responsible for these cues may vary from species to species (van Doorn, 1997; Evanhoe and Galloway, 2002; Rathcke, 2003; Stpczynska, 2003; Blair and Wolfe, 2007; Castro *et al.*, 2008b; Marques and Draper, 2012; Weber and Goodwillie, 2013). In many protandrous species, successful ovule fertilization resulting from pollen deposition leads to floral senescence (Ashman and Schoen, 1997; Arathi *et al.*, 2002; Evanhoe and Galloway, 2002; Blair and Wolfe, 2007). It has been proposed that pollen receipt (completion of female function) is a reliable cue for corolla abscission because by then, pollen removal (completion of male function) is also accomplished, and the maternal plant having completed both the sexual functions can gain the maximum possible benefit (Primack, 1985; Proctor and Harder, 1995; Stpczynska, 2003; Abdala-Roberts *et al.*, 2007; Marques and Draper, 2012). In cases where floral longevity is sensitive to pollen deposition, several factors including the attractiveness of flowers (van Doorn, 1997; Blair and Wolfe, 2007; Glaetli and Barrett, 2008) and placement of floral

organs (Barrett, 2002; Vallejo-Marin and Barrett, 2009) may affect pollen receipt and therefore successful fertilization. In self-compatible species, pollen receipt could potentially be completed during pollen removal with self-pollen depositing on the stigma, but traits such as herkogamy (spatial separation of stigma and anthers) and dichogamy (temporal segregation of male and female phases) significantly reduce this likelihood (Barrett, 2002).

While many angiosperm species readily self-pollinate, not all of them rely on selfing as the predominant reproductive strategy due to associated costs of inbreeding depression (Schemske and Lande, 1985; Goodwillie, 2000; Cheptou and Mathias, 2001; Kennedy and Elle, 2008a; Johnston *et al.*, 2009). In some self-compatible species, herkogamy and dichogamy have been shown to facilitate cross-pollination, with selfing occurring only towards the end of the floral lifespan (Kalisz *et al.*, 1999; Armbruster *et al.*, 2002; Medrano *et al.*, 2005). Such delayed selfing could be a mechanism for reproductive assurance when flowers fail to attract pollinators (Rathcke, 2003; Kalisz *et al.*, 2004; Kennedy and Elle, 2008b; Busch and Delph, 2012). Considering inbreeding depression costs and reproductive assurance benefits, it is natural to expect selfing to be favoured as flowers age, and outcrossing success is progressively reduced (Jarne and Charlesworth, 1993; Busch and Delph, 2012). Some species have floral traits that augment selfing as the corolla begins to abscise, thus supporting the reproductive assurance hypothesis when inbreeding depression is low (Dole, 1990; Kalisz *et al.*, 1999, 2004; Qu *et al.*, 2007; Brys and Jacquemyn, 2011; Evans *et al.*, 2011). Floral longevity, the period for which corolla is maintained on the plant, is therefore an important parameter that determines both outcrossing and selfing and it is known to be influenced by biotic factors such as pollination (Arathi *et al.*, 2002; Castro *et al.*, 2008a; Marques and Draper, 2012) and by abiotic factors such as a stressful maternal environment (Evans *et al.*, 2011).

Resource status of the environment strongly affects maternal trait expression, and when these traits include floral longevity, plant reproductive success is directly affected (Mousseau and Fox, 1998; Arathi *et al.*, 2002; Evanhoe and Galloway, 2002; Delph and Ashman, 2006). In stressful environments, such as under water limitation, the maternal plant exhibits a plastic corolla maintenance response and considerably reduces floral longevity (Arathi *et al.*, 2002). Circumstances such as seasonal extremes of drought or heat result in the resource-stressed maternal plant allocating resources away from floral maintenance and toward offspring production (Ashman and Schoen, 1997; Abdala-Roberts *et al.*, 2007). Therefore, in addition to successful pollination, resource allocation to floral longevity, a maternally regulated trait, would also greatly depend on maternal resource status.

The genus *Collinsia* includes mixed-mating species with the large-flowered ones often being predominantly cross-pollinated, resorting to delayed self-pollination under pollinator limitation (Randle *et al.*, 2009; Kalisz *et al.*, 2012). In mixed-mating species, post-pollination reallocation of maternal resources from floral maintenance into seed production has been argued to be the primary cause for post-pollination corolla senescence. However, sexual conflict, where the male function could manipulate the success of female function via floral longevity could provide an alternative explanation for the evolution of post-pollination corolla senescence (Arnqvist and Rowe, 2005;

Bernasconi *et al.*, 2006; Lankinen *et al.*, 2006). If the corolla retention period in a protandrous species were to be affected by factors extraneous to pollination, such as abiotic stress, modulations to floral longevity could potentially favour one of the sexes, thus exacerbating sexual conflict. *Collinsia heterophylla*, one of the large flowered species in the genus, has been a model system for research on sexual conflict (Lankinen *et al.*, 2006, 2007, 2009; Madjidian and Lankinen, 2009). However, studies addressing the effect of environmental stress on floral traits and reproductive strategies are lacking in this species.

The goal of our study was therefore to investigate the effects of soil water status and pollination schedules on floral longevity and seed production in *C. heterophylla*. This is important in the context of ongoing changes in environmental conditions with unprecedented alterations in precipitation and related phenotypic responses in plants (Nicotra *et al.*, 2010), and can have important implications for our understanding about reproductive success and mating system evolution under extreme drought stress events, similar to what is experienced by endemic populations of *C. heterophylla* (CNRFC, 2012). In our study, we address the following specific questions: (1) How does soil water status affect floral longevity and seed production? (2) How does pollination schedule affect floral longevity and seed production? (3) Do soil water status and pollination schedules interact to affect floral longevity? (4) How does water-stress affect fecundity under autonomous selfing?

MATERIAL AND METHODS

Study system

Collinsia heterophylla (purple Chinese houses; family Plantaginaceae) is a hermaphroditic, annual flowering plant native to California, USA (Newsom, 1929). Protandrous flowers with short stalks are borne in whorls on a raceme, with the corolla of each flower fused to form upper and lower flaring lips. The lower lip of the corolla is typically deep purple, and in rare cases white, while the upper lip is white to light purple (Fig. 1). Each flower has four epipetalous stamens and one pistil. Of the four stamens, two are short and two are long, all



FIG. 1. Floral biology of *Collinsia heterophylla*. A tagged experimental flower with white upper lip (white arrow) and a fully flared lower purple lip (yellow arrow).

of which have red anthers before dehiscence. Protandry in the flowers facilitates the feature of ‘male-first hermaphrodite-later’ during the floral lifespan. The four anthers mature sequentially, beginning from the day after flower opening (Fig. 2). On the day the corolla opens, none of the four anthers has dehisced, and the stigma, which is not yet receptive, is much shorter than the stamens. As in other species of the genus (Armbruster *et al.*, 2002; Kalisz *et al.*, 2012), the first anther in *C. heterophylla* typically dehisces 1 d after the flower opens. The remaining three anthers dehisce one at a time on each subsequent day, making the flowers functionally male for the first 1–3 d. The transition in reproductive role from being exclusively male to that of a hermaphrodite occurs around Day 3 when the style elongates and places the now receptive stigma above the stamens (Armbruster *et al.*, 2002; Lankinen *et al.*, 2007). Flowers of *C. heterophylla* thus exhibit two forms of sexual interference: herkogamy and dichogamy. The onset of stigmatic receptivity is associated with the style elongating past the dehiscent anthers, providing ample opportunity for self-pollen deposition. Pollen competitive ability, however, maintains outcrossing as the dominant mating strategy in this mixed-mating species (Lankinen and Armbruster, 2007). The mature fruits explode, releasing the dark brown seeds.

Experimental layout

Seeds collected prior to 2005, from two naturally occurring populations in Sisar Canyon (34.4°N, 119.1°W) and Wheeler Gorge Campground (34.5°N, 119.3°W) in Ventura County, California, were grown under common greenhouse conditions

to alleviate maternal effects and the seeds obtained from their inter-crossings were used for this study repeated over two years, autumn 2010 and spring 2011. Two to three outcrossed seeds were planted in each of 30 10-cm square pots with similar amounts by mass of Fafard 4P potting soil and maintained at a day temperature of 23 °C, night temperature of 16 °C and a day length of 16 h. These are conditions close to those occurring during the normal growing season of the species in its native environment (USDA NRCS, 2012). Within 7–10 d after germination, seedlings were thinned to one plant per pot and randomly assigned to one of the three watering frequencies, resulting in ten plants in each of the three water treatments: high (watering all days of the week), medium (watering 2 d each week) and low water (watering once each week). The watering frequencies were chosen to reach soil water levels that represent the range that *C. heterophylla* plants can tolerate, with high being close to saturated soil moisture and low being the maximum drying down that was still compatible with plant life. All pots with plants were placed in water for 10 min, sufficient to saturate the soil with water. Soil moisture content was measured 1 week after the seedlings were assigned to treatments, and again when each plant began flowering using the soil moisture probe, EC-5 (Decagon Devices Inc., Pullman, WA, USA) to obtain the volumetric water content (% VWC). A two-way ANOVA, with watering frequency and year as direct effects, and % VWC as the dependent variable, indicated a significant effect of watering frequency on % VWC ($F_{2,155} = 146.01$, $P < 0.0001$). A Tukey post-hoc comparison at $P < 0.0001$ ascertained that soil water status was indeed modulated by watering frequency. High water pots had significantly higher soil water content ($21.7 \pm 3.9\%$ VWC) than the medium and low water

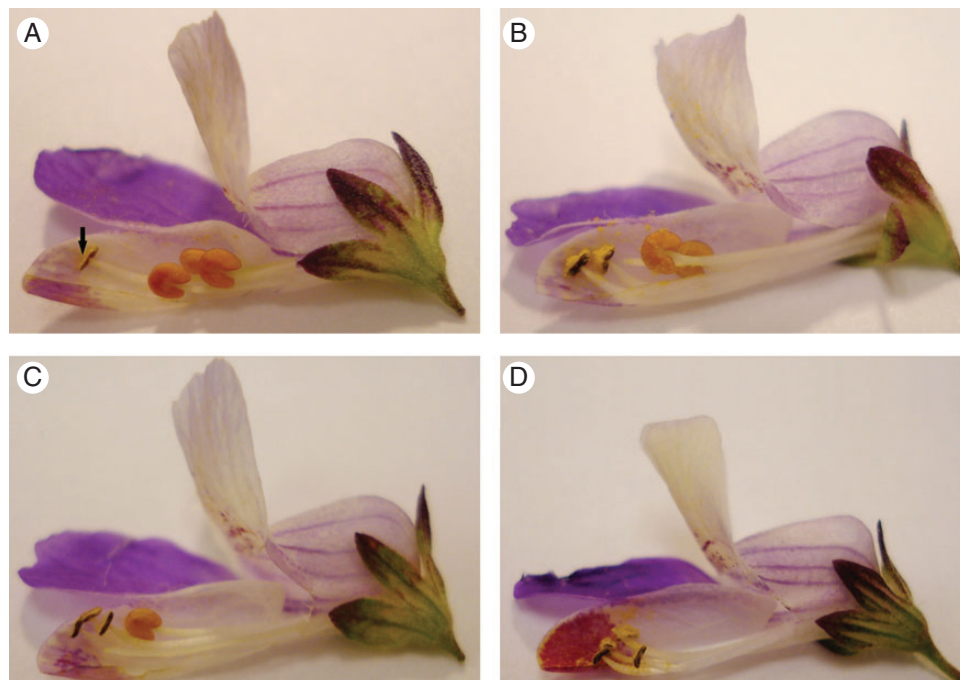


FIG. 2. The sequence of dehiscing anthers demonstrating protandry: (A) one anther (black arrow) dehiscent on Day 1 after flower opening, style shorter than anthers and stigma not receptive; (B) two anthers dehiscent on Day 2 after flower opening, style shorter than anthers and stigma not receptive; (C) three anthers dehiscent on Day 3 after flower opening, style beginning to elongate and stigma gaining receptivity; (D) all four anthers dehiscent on Day 4 after flower opening, style extended beyond the anthers and stigma fully receptive.

ones and medium water pots had higher soil water content ($14.8 \pm 4.3\%$ VWC) than the low water ones ($7.5 \pm 5\%$ VWC).

Longevity experiment

Randomly selected flowers from each of the plants in the three water treatments were subject to three hand-pollination treatments: Early (E), hand-pollinations completed on Days 3 and 4 after anthesis; Middle (M), hand-pollinations on Days 5 and 6 after anthesis; and Late (L), hand-pollinations on Days 7 and 8 after anthesis. Two control treatments per plant in the three water treatments accounted for hand-pollination and emasculation effects: Emasculated Control (Em), flowers were emasculated but not hand-pollinated; and Unmanipulated Control (U), flowers were not handled. Each of the experimental and control flowers was uniquely tagged with coloured tape. One day prior to anthesis, flowers were randomly assigned to the respective hand-pollination treatments or controls (Table 1). The day of anthesis was considered Day 0 and each of the treatment flowers was emasculated 1 d prior to Day 0. To prevent autonomous self-pollination, emasculation was achieved by gently prying apart the petals of a flower and removing all four anthers using a pair of dissection forceps. The stigma received pollen from one flower of a different plant within the same water treatment to minimize pollen donor effects. Pollen from the single donor flower was collected on a sterilized microscope slide using dissection forceps, and hand-pollinations were accomplished by dipping the recipient stigma into the pollen on the slide such that the stigmatic surface was covered with pollen. Experimental and control flowers were monitored daily for corolla wilting or abscission. Floral longevity was determined as the number of days from flower opening to abscission or wilting. Developed ovaries were collected at fruit maturity, as evident from the beginnings of a split in the sutures of the fruit coat. Fruits were allowed to air dry before determining seed number per fruit.

Statistical analyses

Analysis of floral longevity was performed by completing a two-way ANOVA with soil water status and hand-pollination schedules as independent variables, year as the random effect and floral longevity as the dependent variable. Analyses of seed numbers were performed by executing (1) a two-way ANOVA within the hand-pollinated treatments (E, M and L), with soil water treatment and hand-pollination schedules as independent variables, year as the random effect and seed production as the dependent variable and (2) a one-way ANOVA comparing the effect of soil water treatment on seed numbers in the

TABLE 1. Number of experimental flowers for each pollination schedule and water treatments

		Soil water treatments		
		Low	Medium	High
Pollination schedules (days after anthesis)	Early: E (3–4)	30	29	33
	Middle: M (5–6)	30	29	32
	Late: L (7–8)	30	28	31
Procedural controls	Emasculated: Em	18	18	18
	Unmanipulated: U	18	18	19

Unmanipulated Control (U). All statistical analyses were executed in IBM SPSS Statistics Version 21.0 (IBM Corp., Armonk, NY, USA), which incorporates Bonferroni-corrected *P*-values for multiple pairwise comparisons.

RESULTS

Floral longevity of hand-pollinated flowers

Our results indicate that pollination schedules and soil water status had significant direct and interactive effects on floral longevity in *C. heterophylla* (Table 2). Pollinations early in the floral lifespan reduced corolla retention period significantly over hand-pollinations late in the floral lifespan while the longevity of flowers pollinated during the middle of the floral lifespan was not different from either early or late hand-pollinations (Fig. 3A). Analysis of the interaction effect of soil water status and pollination schedule on floral longevity indicates that flowers under low soil moisture hand-pollinated early in the floral lifespan retained their corolla for just over 6 d, which is a significant reduction in longevity as compared with late-pollinated flowers in any water status (Fig. 3B). The highest average longevity of over 9 d was seen in flowers receiving hand-pollinations late in the floral lifespan in all the three watering regimes (Fig. 3B). Year did not affect floral longevity.

Floral longevity of control flowers

Pollination significantly reduced corolla retention periods, as evidenced by the emasculated and unmanipulated flowers exhibiting significantly higher longevity than hand-pollinated flowers (Fig. 3A). Soil water status affected the longevity of the control flowers, with high water plants maintaining corollas for significantly longer periods than low water plants (Fig. 3B). Lack of emasculation and hand-pollinations resulted in the largest values for floral longevity across all water treatments

TABLE 2. Effects of soil water and pollination schedules on floral longevity and seed number per fruit in *Collinsia heterophylla*

Source	d.f.	Mean SS	<i>F</i> ratio	<i>P</i>
Floral longevity				
Year	1	9.7	0.9	0.33
Soil water status	2	57.5	5.6	0.004
Pollination schedules	4	1202.6	117.8	<0.0001
Soil water status × pollination schedules	8	23.5	2.7	0.007
Error	351	10.2		
Seed number per fruit (pollinated)				
Year	1	183.4	6.0	0.02
Soil water status	2	153.9	5.1	0.007
Pollination schedules	2	29.9	0.9	0.3
Soil water status × pollination schedules	4	28.3	0.9	0.4
Year × pollination schedules	2	7.8	0.3	0.8
Error	247	30.5		
Seed number per fruit (unmanipulated)				
Year	1	0.4	0.03	0.9
Soil water status	2	38.70	3.08	0.05
Error	48	12.53		

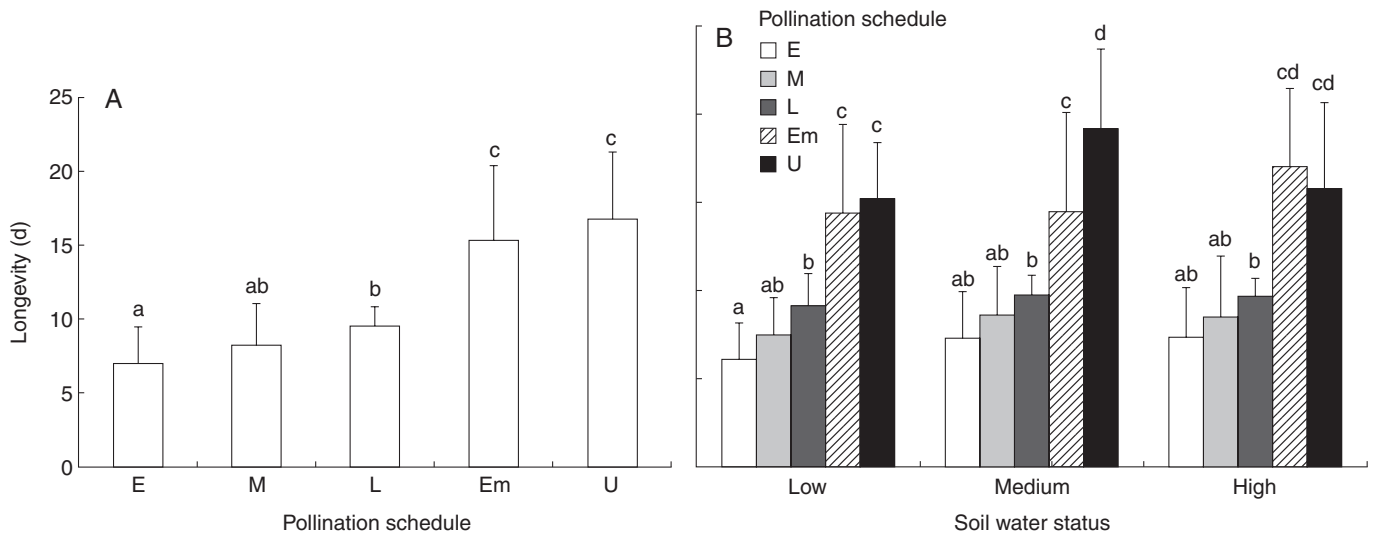


FIG. 3. (A) Pollination schedules significantly influenced floral longevity in *C. heterophylla*. Each bar represents the average days to corolla abscission (\pm s.d.). Bars with different letters indicate significant differences in floral longevity among pollination treatments by a Tukey *post-hoc* comparison at $P < 0.05$. (B) Interactive effect of soil water and pollination schedules on longevity. The x-axis refers to different soil water treatments and individual bars refer to different pollination treatments described in Table 1 and indicated in the key: early (E), middle (M), late (L) hand-pollinations, emasculated (Em) and unmanipulated (U) controls. Bars with different letters indicate statistically significant differences across pollination schedules within a soil water treatment by a Tukey *post-hoc* comparison at $P < 0.05$.

with intact, unmanipulated flowers in medium water retaining their corollas for over 19 d. While the corolla retention periods of unmanipulated flowers in medium water plants were not significantly different from the corresponding values from high soil moisture plants, they were significantly higher than those in plants grown under low soil moisture (Fig. 3B).

Seed production in hand-pollinated and control flowers

Soil water status significantly influenced seed production both in hand-pollinated treatment and in the unmanipulated control (Table 2). Low soil water consistently resulted in a significant reduction in per-capita seed production in all the three hand-pollinated treatments (Fig. 4A). Seed production from autonomous self-pollination in unmanipulated flowers was significantly higher in plants growing under high soil water (Fig. 4A). Per-capita seed production ranged from 9–10 seeds in hand cross-pollinated flowers to two seeds in unmanipulated, autonomously selfed flowers. A non-significant difference between per-capita seed production across pollination schedules suggests that the timing of pollination in the floral lifespan does not determine fecundity. However, seed production by autonomous selfing was marginal at best, as indicated by the significantly lower per-capita seed numbers in the unmanipulated flowers (2.2 ± 3.5 seeds per fruit; Fig. 4B), implying that outcrossing is the predominant mating strategy. Our method of emasculating was effective, as evidenced by the result that emasculated controls did not produce any seeds in a pollinator-free greenhouse. While year did have a significant effect on per-capita seed numbers in pollinated flowers, its effect was not significant in unmanipulated flowers.

DISCUSSION

Floral longevity in *Collinsia heterophylla* appears to be a plastic trait modulated by biotic factors such as when pollination occurs

during the floral lifespan, as well as abiotic factors such as soil moisture status. Our results parallel the model of optimal floral longevity, which predicts reduced periods of corolla retention when the cost of floral maintenance and fitness accrual rates therein are both high (Schoen and Ashman, 1995). If the primary function of corolla in angiosperms is associated with pollinator attraction, once pollination is accomplished, resource flow to the corolla may be minimized leading to corolla abscission. Our observations suggest that corolla abscission following pollination can be exacerbated by drought conditions. While extending the period of corolla retention beyond pollination may result in some additional benefits for the plant (van Doorn, 1997), under low soil moisture the cost of maintaining a turgid corolla, which continues to lose water by transpiration, significantly outweighs the benefits (Galen, 2000). Resource constraint appears to determine the duration of corolla retention in *C. heterophylla*, favouring abscission soon after pollination, as evidenced by substantially reduced floral longevity in the low soil moisture and early pollination treatment. However, high soil water conditions that could be considered as an indication of a resource surplus did not result in increased floral longevity, further supporting our hypothesis that corolla abscission is closely tied to pollination. Although flowers are known to be resource sinks (Galen, 2000), excessive availability of resources does not necessarily enhance the period of flower retention beyond the completion of the flower's sexual function.

In a protandrous species such as *C. heterophylla*, in which the stigma remains receptive for a few days after anthers have shed pollen, maintaining flowers post-pollination could prolong the period for accrual of female fitness and enhance the diversity of distinct pollen genotypes on the stigma by an extended period for pollinator visitation (van Doorn, 1997; Delph and Havens, 1998). Extrapolating the findings of Lankinen *et al.* (2007) that delayed stigmatic receptivity facilitates pollen competition on the stigma, we propose that the likelihood of pollen competition could potentially be strengthened, if the period of corolla

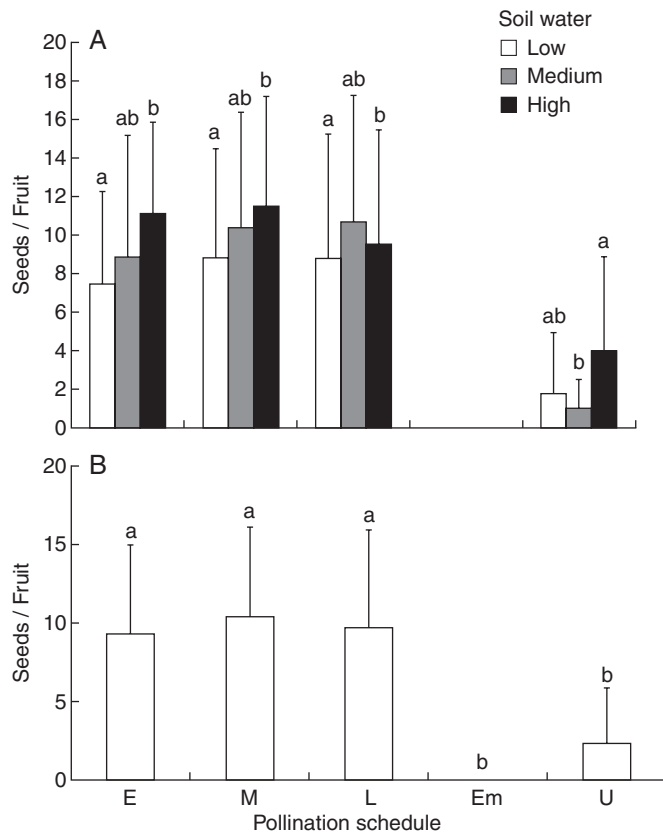


FIG. 4. (A) Effect of soil water status on per-capita seed set. The x-axis denotes different pollination schedules described in Table 1: early (E), middle (M), late (L) hand-pollination treatments as well as emasculated (Em) and unmanipulated (U) controls. The individual bars refer to soil water status: low, medium and high soil water, as indicated in the key. Per-capita seed set is compared within each hand-pollination treatment and unmanipulated control across soil water status and the letters on the bars indicate respective significant differences by a Tukey *post-hoc* comparison at $P < 0.05$. (B) Autonomous selfing resulted in significantly reduced per-capita seed set. Bars with different letters indicate significant differences in seed number per fruit at $P < 0.05$ in a Tukey *post-hoc* comparison.

retention were to be extended. Studies in other species also allude to enhanced offspring fitness, as possible benefits of increased stigmatic pollen competition (Mulcahy and Mulcahy, 1975; Davis *et al.*, 1987; Delph and Havens, 1998; Mazer *et al.*, 2010; Winsor *et al.*, 2000; Armbruster and Rogers, 2004, and references therein). Maintaining pollinated flowers therefore could allow for the possibility of multiple pollinator visits, increasing the number of pollen donors and the resultant benefits of pollen competition (Kalla and Ashman, 2002). While the above studies demonstrate the benefits of increasing floral lifespan, other studies indicate that pollination triggers irreversible changes in physiology, resulting in corolla abscission, within a few hours to a few days after pollen receipt (van Doorn, 1997; Abdala-Roberts *et al.*, 2007; Blair and Wolfe, 2007; Clark and Husband, 2007; Castro *et al.*, 2008a). Our study supports the hypothesis that pollination triggers changes that lead to corolla abscission irrespective of the soil water status. Not only did the unmanipulated flowers in our experiments retain corollas longer than the flowers that received hand-pollinations early in the floral lifespan, they also maintained flowers 5–8 d longer than those that received hand-pollinations late in the floral lifespan.

Given that plants frequently operate under resource constraints, the life history of an annual plant limits the duration for which an unpollinated flower can maintain its corolla. In many plant species, autonomous selfing may be favoured towards the end of the floral lifespan, in order to ensure some amount of reproductive success even as resource flow towards such older flowers may continue to deplete (Dole, 1990; Kalisz *et al.*, 1999; Arathi *et al.*, 2002; Duan *et al.*, 2010; Sicard and Lenhard, 2011; Busch and Delph, 2012). While floral longevity is likely to be highly reduced in predominantly selfing species as compared with closely related outcrossing species (Weber and Goodwillie, 2013), flowers retain their attractive structures well into the floral lifespan in *C. heterophylla*, despite ample opportunities to self-pollinate, reiterating that reproductive assurance by delayed selfing is only likely when pollinators are limiting (Dole, 1990; Arathi *et al.*, 2002; Kalisz *et al.*, 2004; Elle *et al.*, 2010). The main purpose of the corolla is for pollinator attraction and successful cross-pollination. Reduced allocation to floral traits that facilitate pollinator attraction is predicted when selfing becomes the predominant breeding mode, due to the redundancy of attractive floral parts, allowing selection to favour reduced corolla retention and, in extreme cases, a highly reduced corolla (Goodwillie *et al.*, 2009). Our results thus lend credence to the growing body of evidence that *C. heterophylla*, like many other large-flowered species in the genus, is predominantly cross-pollinated, and at best a delayed selfer (Mayer *et al.*, 1996; Kalisz *et al.*, 1999, 2004, 2012). The exact timing and mechanism for selfing have not been described in *C. heterophylla* but similarity in floral structure allows us to predict that mechanisms similar to that in the other species of the genus may be at play.

In hermaphroditic flowering plants, fitness accrues from both the male function of pollen export, and the female function of pollen receipt and ovule fertilization. Sexual conflict between these two functions could determine the expression of floral traits that benefit one of the sexes at a significant cost to the other (Fetscher, 2001; Prasad and Bedhomme, 2006; Bedhomme *et al.*, 2009). Floral longevity in *C. heterophylla* could directly relate to such a conflict, as early corolla abscission allows pollen export (male function) in this protandrous species to be accomplished before stigma reaches peak maturity. While there exists a possibility of early deposition of cross-pollen, the benefits of such cross-pollination are likely to be minimal (Lankinen and Kiboi, 2007). Fulfilling the female function (pollen receipt) in this cross-pollinated species, which also represents an indication of successful fecundity for the maternal parent therefore, requires that corolla retention be extended beyond the period of anther functionality in the flower. However, if abiotic stress, such as drought, significantly reduces floral lifespan then the associated advancement of delayed selfing would allow for the fulfilment of female function only via saturation of the stigma with self-pollen while male function may be less compromised given the continued likelihood of pollen export from the early dehiscing anthers. Ongoing studies (H. S. Arathi, Colorado State University, CO, pers. comm.) on the effects of abiotic stress on floral traits in *C. heterophylla* indicate that patterns of anther dehiscence are not adversely affected, supporting our hypothesis that the male function of pollen export is not compromised under stress. Reduced floral lifespan that largely benefits the male sex could therefore aggravate sexual conflict by limiting opportunities for outcrossed pollen deposition on the

stigma and increasing costs of inbreeding depression (Lankinen and Armbruster, 2007), constraining the adaptive significance of delayed stigmatic receptivity in this species (Lankinen *et al.*, 2007). Unless there is a parallel advancement of stigmatic receptivity under abiotic stress, floral longevity could become a male-controlled trait in this protandrous hermaphrodite. Plants have been generally shown to rely on pollen receipt rather than pollen export as a cue for corolla senescence (Ashman and Schoen, 1997; Arathi *et al.*, 2002; Blair and Wolfe, 2007; Castro *et al.*, 2008a; Marques and Draper, 2012), which can be argued as a mechanism favoured to minimize runaway effects of sexual conflict in hermaphrodites (Arnqvist and Rowe, 2005; Bernasconi *et al.*, 2006; Lankinen *et al.*, 2006).

Discussion on the functional significance of the corolla and its longevity is incomplete without information on reproductive success, i.e. per-capita seed production. In our study, seed numbers were affected by pollination and soil moisture status. Previous research with *C. heterophylla* has shown that early pollination results in a reduced number of seeds per capsule (Lankinen and Kiboi, 2007). This directly contrasts with our finding that timing of pollination did not affect seed production. The discrepancy may lie in the timing of 'early' pollinations in Lankinen and Kiboi (2007) where pollinations were done on Day 2 after flower opening, which is before the stigma becomes receptive (Lankinen *et al.*, 2007). In contrast, our earliest hand-pollination treatments were designed to coincide with stigmatic receptivity and probably resulted in higher seed numbers than those observed in Lankinen and Kiboi (2007). While significant differences in seed production between hand-pollinated flowers and emasculated control flowers are to be expected, fewer seeds in the unmanipulated controls support the increased efficacy of cross pollen in *C. heterophylla*, a predominantly outcrossing species (Mayer *et al.*, 1996; Kalisz *et al.*, 1999, 2004). The reduced seed numbers under autonomous selfing could be due to herkogamy-mediated limitations to self-pollen deposition as the receptive stigma is situated beyond the stamens (Fig. 2D) and/or the possibility of reduced pollen grain viability with pollen age (Arathi *et al.*, 2002). In addition, high amounts of inbreeding depression, known to be prevalent in *C. heterophylla*, could largely limit fecundity by autonomous selfing in this species (Mayer *et al.*, 1996).

Taken together, our results on the variation in corolla retention and seed production as a function of pollination and soil moisture demonstrate the important role of the maternal plant in allocating resources from one crucial function (e.g. corolla maintenance) to the next (e.g. seed production) through its life cycle. Extending the concept of hierarchical allocation, we argue that once the function of an organ, in our case that of the flower, has been accomplished, plants may be favoured to route resources to the next process in the life cycle, that of seed production (Obeso, 2004). With little information on the genetic control of floral longevity, we can only suggest that the duration of corolla retention has a strong potential to influence the evolution of mating system in a species. Given that flowers are costly structures to maintain (Primack, 1985; Galen, 2000), and that maternal control of floral traits such as herkogamy and dichogamy may respond differently to a limiting resource, water limitation has the potential for multiple and synergistic effects on reproductive output. Soil moisture is a critical resource for reproductive success, making

it important to understand the long-term ramifications that water shortage can have on plant fecundity and population size.

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