

Floral closure induced by pollination in gynodioecious *Cyananthus delavayi* (Campanulaceae): effects of pollen load and type, floral morph and fitness consequences

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• **Background and aims** Pollination-induced floral changes, which have been widely documented in flowering plants, have been assumed to enhance the plant's reproductive success. However, our understanding of the causes and consequences of these changes is still limited. Using an alpine gynodioecious species, *Cyananthus delavayi*, we investigated the factors affecting floral closure and estimated the fitness consequences of floral closure.

• **Methods** The timings of floral closure and fertilization were determined. The effects of pollen load, pollen type (cross- or self-pollen) and floral morph (female or perfect flower) on the occurrence of floral closure were examined. Ovule fertilization and seed production were examined to investigate the causes and consequences of floral closure. Flowers were manipulated to prevent closing to detect potential benefits for female fitness.

• **Key Results** Floral closure, which could be induced by a very low pollen load, occurred within 4–7 h after pollination, immediately following fertilization. The proportion of closed flowers was influenced by pollen load and floral morph, but not by pollen type. Floral closure was more likely to occur in flowers with a higher proportion of fertilized ovules, but there was no significant difference in seed production between closed and open flowers. Those flowers in which closure was induced by natural pollination had low fruit set and seed production. Additionally, seed production was not influenced by closing-prevented manipulation when sufficient pollen deposition was received.

• **Conclusions** The occurrence of floral closure may be determined by the proportion of fertilized ovules, but this response can be too sensitive to ensure sufficient pollen deposition and can, to some extent, lead to a cost in female fitness. These results implied that the control of floral receptivity by the recipient flowers does not lead to an optimal fitness gain in *C. delavayi*.

Key words: *Cyananthus delavayi*, female fitness, floral closure, floral longevity, gynodioecy, pollination, post-pollination phenomenon, sexual conflict.

INTRODUCTION

The duration of floral lifespan (floral longevity) plays an important role in the reproduction of flowering plants through its effect on both pollen dispersal and pollen receipt (Primack, 1985; Galen *et al.*, 1986; Harder and Wilson, 1994; Bingham and Orthner, 1998; Ishii and Sakai, 2000). A relatively extended floral longevity may be beneficial for ensuring sufficient pollen deposition, facilitating pollen dispersal, promoting competition among pollen grains and increasing the proportion of cross-pollination (Proctor and Harder, 1995; Paschke *et al.*, 2002; Bernasconi, 2003; Rathcke, 2003; Bernasconi *et al.*, 2004). However, pollination-induced reduction of floral longevity has been widely documented: the flower's lifespan is significantly shortened after pollination in numerous species (reviewed by Primack, 1985). In some cases, floral form changes (flowers wither or close) soon after pollen deposition (Primack, 1985).

A widely accepted explanation for post-pollination changes in floral form is that, from the perspective of the pollen

recipient, optimizing floral longevity requires a cost–benefit trade-off. Maintaining flowers in a functional state requires resource that could otherwise be allocated to other processes (e.g. production of seed or new flowers) (Webb and Littleton, 1987; Ashman and Schoen, 1997; van Doorn, 1997; Abdala-Roberts *et al.*, 2007). It has been suggested that optimal floral longevity is a compromise between maximizing reproductive success and minimizing further investment in flower maintenance (Schoen and Ashman, 1995; Ashman and Schoen, 1996). It has thus been assumed that once reproductive function is complete, a flower should wither as soon as possible to minimize the costs of flower maintenance (van Doorn, 1997; Lankinen *et al.*, 2006; Clark and Husband, 2007).

More recently, sexual conflict, a theory originating from animal studies, has been applied to explain post-pollination phenomena such as flower wilting (Lankinen *et al.*, 2006). Based on a game-theoretical model, Lankinen *et al.* (2006) suggested that floral changes may be manipulated by the pollen donor rather than the pollen recipient as a means of

securing paternity. If this floral change decreases the chance of additional pollen deposition, the paternity of pollen donors that supply the first pollen to reach the flower can be secured under conditions of intense pollen competition at the expense of female fitness (Lankinen *et al.*, 2006). However, this hypothesis has only been tested by limited empirical studies (Lankinen and Kiboi, 2007; Madjidian and Lankinen, 2009).

To understand the adaptive significance of floral changes, it is necessary to establish the causes and fitness consequences of these changes. The timing of floral changes may throw light on the causes of these responses, i.e. to determine whether they are triggered by pre-zygotic events (e.g. pollen deposition) or by post-zygotic events (e.g. fertilization). But it has been reported only in a few studies, and these gave contrasting results, with floral closure occurring either before or after fertilization (He *et al.*, 2006; Clark and Husband, 2007). The factors that may have influence on female fitness were suggested to have effects on floral changes. Pollen quantity may be the most important factor. It has been shown that increased pollen load caused the responses to occur more rapidly, leading to earlier termination of floral lifespan (Evanhoe and Galloway, 2002; Giblin, 2005; Clark and Husband, 2007). Pollen type (self- or cross-pollen) was found to affect floral longevity in some cases but not in others (Sato, 2002; Gibbs *et al.*, 2004; Clark and Husband, 2007). Floral morph (e.g. perfect or female flowers in this study), however, is a factor that has never been considered in previous studies, since most of them used hermaphroditic plants. If these factors have effects on floral changes, they may further influence a plant's reproductive fitness by changing floral longevity. As far as we know, the only report that has considered the effect of floral changes on reproductive fitness has been presented by He *et al.* (2006), who suggested the closed but persisting corolla is beneficial for seed set of pollinated flowers. However, their results were derived from a sufficient pollen-deposition condition. It is still unclear whether floral changes always lead to positive fitness consequences, especially when insufficient pollen is received for seed production.

Permanent floral closure following pollination is found in *Cyananthus delavayi*, an alpine gynodioecious species endemic to south-west China. In our preliminary study, flowers in which closure was induced by natural pollination were common in the field, but the fruit set and seed production of these closed flowers were very low. In the present study, we investigated the causes of this floral closure and estimate its consequences for female reproductive success. Specifically, the effects of pollen load, pollen type (cross- or self-pollen) and floral morph (female or perfect flower) on floral closure were examined. Ovule fertilization and seed production were then examined to estimate the consequences of floral closure mediated by the above-mentioned factors. We hypothesized these factors may have effects on floral closure through their influence on female fitness, and floral closure may further influence the future reproductive fitness of the plant. The following questions were addressed. (1) Is floral closure triggered by fertilization? (2) Is floral closure influenced by pollen-load size, pollen type and/or floral morph, and what is the determinant cause of the occurrence of floral closure? (3) What is the minimum pollen load necessary to induce flower closure and

how does it compare, in terms of its effect on female fitness, with higher pollen loads? (4) Does floral closure provide any benefits for ovule fertilization and seed production?

MATERIALS AND METHODS

Study species and locality

Cyananthus delavayi Franch. (Campanulaceae) is a prostrate herbaceous perennial, endemic to the alpine area in the south-west of China (Hong and Ma, 1991). The growth habit of this species is characterized by caespitose stems with small leaves. It produces showy violet-blue tubular-campanulate flowers, with five corolla lobes. The flowering season is usually from mid-August to late September. The number of flowers produced per plant ranges from dozens to hundreds depending on the age of the plant. The population of *C. delavayi* includes two plant morphs, females and hermaphrodites, and is therefore gynodioecious (Fig. 1A, B). Preliminary work showed that the female frequency is 53.25% in the population used in this study. Like other species in the Campanulaceae, perfect flowers in *C. delavayi* are protandrous and have a secondary pollen presentation mechanism. Although selfing within an individual flower is avoided by complete protandrous behaviour, geitonogamous self-pollination is possible between flowers of different gender-phases within the same hermaphroditic plant. This species is partially self-incompatible, with selfing resulting in lower seed set than crossing (Y. Niu and H. Sun, unpubl. res.). Halictid bees (*Halictus* sp.), bumble bees (*Bombus richardis* and *B. festivus*) and hoverflies are the main visitors to *C. delavayi*, but the visitation rate is relatively low (Y. Niu and H. Sun, unpubl. res.). Under natural conditions, the floral longevity of both floral morphs is about 4–5 d (Y. Niu and H. Sun, unpubl. res.). Two types of floral closure have been found in this species: one is temporary closure, which is induced by environmental conditions (temperature or humidity) and occurs every evening, and the other is pollination-induced permanent closure, which occurs within several hours of successful pollination and is the focus of the present study. When permanent closure occurs, flowers are no longer able to receive visits from pollinators.

The Hengduan Mountains in south-west China are the core area for the differentiation of the genus *Cyananthus* (Hong and Ma, 1991). Our field study site (27°54'N, 99°38'E, elevation 3355 m a.s.l.) is located on the east side of Napa Lake, Shangri-la County (formerly Zhongdian County), Diqing plateau, in the north-west of Yunnan province. It is in the native habitat of *C. delavayi* and includes bare land and sparse grassland protected from grazing. The typical vegetation includes species of the following genera: *Anaphalis*, *Pedicularis*, *Salvia*, *Lomatogonium*, *Sedum* and *Onosma*. During the period from 2004 to 2008, the mean annual air temperature recorded at the nearest meteorological station (27°50'N, 99°42'E, elevation 3279 m a.s.l.) was 6.7 °C and the mean annual precipitation was 587.8 mm. The present study was conducted during the flowering seasons in 2009 and 2010.

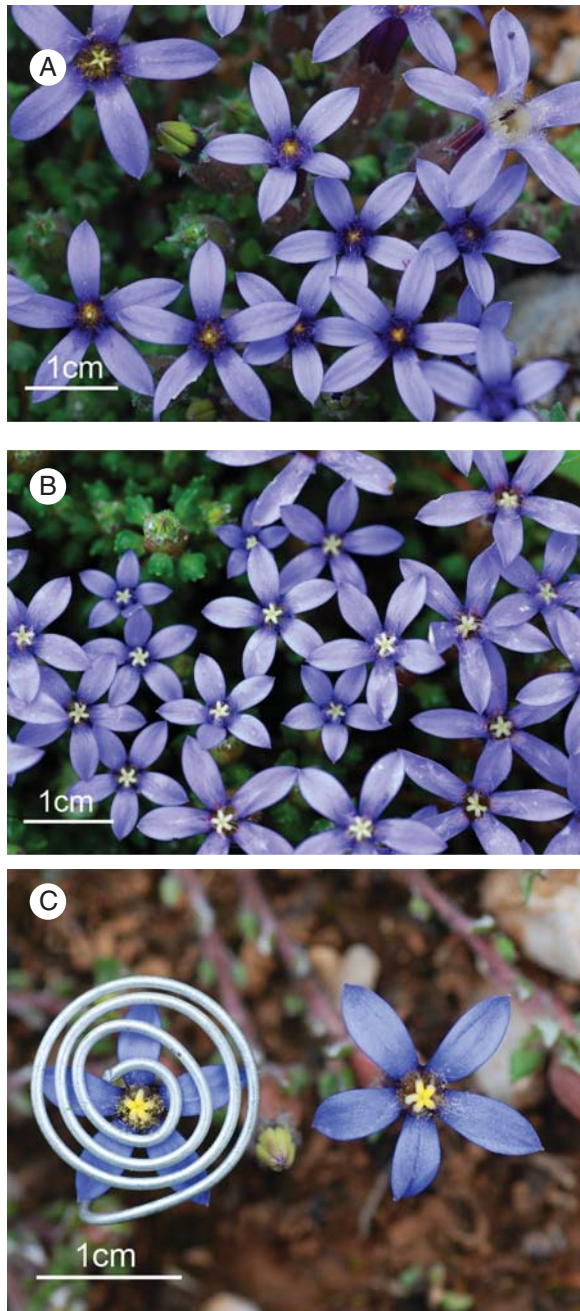


FIG. 1. Hermaphroditic (A) and female (B) plants of *Cyananthus delavayi* (Campanulaceae) in a natural population. Corolla lobes are prevented from closing after pollination in treatment group, using a fine wire ring (C).

Time until floral closure

To investigate when floral closure occurs and whether it is triggered before, or as a consequence of, fertilization, we recorded the time lapse from pollination to floral closure and examined how many ovules had been fertilized when floral closure occurred. Thirty female flowers from ten female plants (three flowers from each plant) and 22 perfect flowers from 11 hermaphroditic plants (two flowers from each plant) were pollinated with sufficient cross-pollen between 1000 and 1100 h on a sunny day, thus ensuring enough time to

distinguish permanent closure from temporary closure, which usually occurs after 1900 h. The experimental plants were covered with nylon nets to prevent natural pollen deposition before hand-pollination. Monitoring was conducted at 1-h intervals for the first 4 h, and then at 10-min intervals until the flower closed. A flower was defined as closed when three of the five corolla lobes folded and touched together. The time of floral closure was calculated as the length of time from pollination to floral closure (in hours). When closure occurred, the styles were collected immediately and preserved in FAA (formalin–acetic acid–alcohol), and subsequently stained with Aniline Blue Lab according to the technique described by Dafni (1992). A fluorescence microscope was used to observe the status of pollen tube growth and to count the number of fertilized ovules. An independent samples *t*-test (SPSS 13.0) was carried out to determine whether there were differences between female and perfect recipients with respect to the time of floral closure and the mean numbers of fertilized ovules when closure occurred.

To locate the time of floral closure on the timeline of fertilization events (i.e. how many ovules had been fertilized at that time) and to evaluate if some ovules were fertilized after floral closure, we examined the number of fertilized ovules in recipient flowers at different times (from 2 to 48 h) after pollination. An additional 118 unopened female flowers from 12 plants were randomly divided into seven groups and hand-pollinated with cross-pollen on the first day of anthesis. After that, they were collected 2, 4, 6, 8, 16, 24 and 48 h after pollination. For each group, the status of pollen-tube growth was observed using the method described above and the number of fertilized ovules was counted. Curve estimation (SPSS 13.0) was used to analyse the relationship between sampling time and the number of fertilized ovules.

The effects of pollen-load size on floral closure and female fitness

A series of experiments was designed, on both female and perfect flowers, to examine the effects of pollen load on floral closure and to estimate the female fitness of the flowers. We hypothesized that pollen-load size may have effects on floral closure through their influence on female fitness.

Preliminary studies indicated that a very small amount of pollen is sufficient to induce floral closure, and thus we loaded stigmas with different, but very low, amounts of pollen to test the effects on floral closure. For female recipients, nine recipient flowers from each female plant were randomly chosen and evenly divided into three groups to receive 10, 20 or 30 pollen grains. Twenty-four female plants were involved in these experiments, and 72 female flowers were pollinated in total at each pollen-load level (216 flowers at all three levels). For perfect recipients, 12 flowers from each hermaphroditic plant were selected and randomly divided into two groups, to receive either cross- or self-pollen (see details in the following section on the effect of pollen type). For each group, the six flowers were evenly and randomly assigned to receive 10, 20 or 30 grains. In total, 15 plants were used in these experiments and 30 perfect flowers were involved for each pollen load by pollen type combination. Each recipient flower received cross-pollen from a

single cross-donor. Self-pollen was collected from the male-phase flowers produced by the same plant. Application of low pollen loads was achieved as follows: a small number of pollen grains were carefully dispersed onto a clear microscope slide from the outside surface of the folded stigma lobes. Redundant grains were then swept away using fine cotton tips until the residual grains on the slide reached the required number (10, 20 or 30). A portable stereomicroscope ($\times 60$ magnification) allowed us to check the exact number of grains on the slide in field. The slide was inverted and applied to the stigma, transferring the pollen grains. Hand pollination was conducted between 1300 and 1500 h on the first day of anthesis, before that the plants were covered with nylon nets to prevent natural pollen deposition. The status of each flower was recorded at 1400 h on the second day. Additional pollen deposition was prevented by covering the whole plant with nylon net after pollination. The status (closed or open) of these pollinated flowers was recorded the day after pollination. Fruits were collected 4 weeks after pollination to estimate ovule fertilization and seed production. Fruits from some pollinated flowers were lost before the collection date because of wind, rain or consumption by herbivores. Since ten grains were not sufficient for perfect flowers to set fruits, most fruits of these flowers were lost. For these flowers, the data related to female fitness were not obtained. Data were only included in analyses for flowers where both flower status and female fitness were available (163 female flowers and 98 perfect flowers).

In the present study, we did not use the speed of floral closure as an index to estimate the effects of pollen-load size on floral closure for two reasons. First, after having received the same amount of pollen grains (at low pollen-load size), some flowers closed within a few hours while others remained open as long as the unpollinated flowers (Y. Niu and H. Sun, unpubl. res., see discussion). Secondly, although pollen-load size had a significant effect on the speed of floral closure (based on an ANOVA analysis), a lower pollen-load size, did not always result in a slower speed of floral closure (Y. Niu and H. Sun, unpubl. res.). Therefore, we used the proportion of closed flowers (PC) to estimate the effect of pollen load on floral closure. This proportion was calculated as the number of closed flowers divided by the total number of flowers pollinated.

To determine the causes of floral closure and to estimate the female fitness of the flowers, we used pollen density (PD; the number of pollen grains on a stigma divided by the number of ovules), the number of fertilized ovules per fruit (NF; includes both viable seeds and aborted seeds), the proportion of fertilized ovules per fruit (PF; the number of fertilized ovules divided by the number of total ovules), seed number per fruit (SN; the number of viable seeds per fruit) and seed set per fruit (SS; the number of viable seeds divided by the number of total ovules) as indices to estimate ovule fertilization and seed production. Of these indices, PD is involved in pre-zygotic processes, whereas the others are involved in post-zygotic processes. It seems redundant to examine PD when pollen-load size is controlled. However, given that the number of ovules varies among recipient flowers, we thought it is necessary to verify that our operation on pollen-load size had increased PD significantly as we had expected.

The effects of pollen-load size on the proportion of closed flowers were examined using a 2×3 contingency table for female recipients, and a 2×2 contingency table for perfect recipient flowers (JMP 6.0). The data of ovule fertilization and seed production were compared among different pollen-load sizes using one-way ANOVA (SPSS 13.0). PD values were log-transformed and the other values were sqrt-transformed for analyses to meet the assumptions of ANOVA.

The effects of pollen type on floral closure and female fitness

The proportion of closed flowers and the data of ovule fertilization and seed production were compared between perfect recipients that had received cross- and self-pollen. We hypothesized that pollen type may have effects on floral closure through their influence on female fitness. All data involved were obtained from the experiments mentioned in the last section (49 flowers with cross-pollination and 49 flowers with self-pollination). The differences in the proportion of closed flowers between two pollen types were examined using a 2×2 contingency table at pollen-load sizes 20 and 30 (JMP 6.0). An independent samples *t*-test (SPSS 13.0) was used to examine the difference in female fitness between two pollen types at pollen-load sizes 20 and 30.

The effects of floral morph on floral closure and female fitness

The proportion of closed flowers and the data of ovule fertilization and seed production were compared between female and cross-pollinated perfect recipients at each pollen-load size. We hypothesized that pollen type may have effects on floral closure through their influence on female fitness. The related data were obtained from the experiments mentioned in the last two sections (111 female flowers and 49 perfect flower), and analysis methods were described therein.

The differences in female fitness between closed and open flowers under the same condition

To answer the question why some flowers closed after pollination while others, under the same conditions, maintained open (i.e. what is the determinant fact to the occurrence of floral closure), and to investigate whether closed flowers produce more seed than the open ones, an independent samples *t*-test (SPSS 13.0) was used to examine the difference in ovule fertilization and seed production between closed and open flowers under the same condition. Binary logistic regressions (SPSS 13.0) were used to examine further the relationship between female fitness and the occurrence of floral closure for each pollen-load size, with flower status (closed or open) as the independent variable and indices of female fitness as the covariates.

Fitness consequences of floral closure under natural and manipulated conditions

First, to investigate whether female fitness was ensured before flowers closed, flowers in which closure was induced by natural pollination were tagged in field and collected

when fruits matured. Fruit set was calculated and compared between the two floral morphs using a 2×2 contingency table. For fruits with seeds, the number of seeds per fruit was counted and a comparison between the two morphs was made using an independent samples *t*-test.

Secondly, we investigated whether floral closure *per se* provides extra benefits for seed production when pollen deposition is sufficient, on both female and perfect flowers. A pair of flowers from each plant was randomly chosen and pollinated with sufficient cross-pollen. After that, one flower of each pair was allowed to close freely after pollination. The other had a fine wire ring applied to the corolla to prevent floral closure, and the foot of the wire was inserted into the ground to fix it in place (Fig. 1C). This method prevented flowers from closing without causing any damage. Thirty pairs of female flowers and 22 pairs of perfect flowers were involved in this experiment. The difference in seed number per fruit between groups was analysed using a paired samples *t*-test (SPSS 13.0).

RESULTS

Time until floral closure

Permanent floral closure occurred between 4.2 and 6.5 h after pollination in female flowers and between 5.0 and 7.0 h after pollination in perfect flowers. The average time from pollination to floral closure in female flowers [5.38 ± 0.13 h (mean \pm standard error), $n = 30$] was significantly shorter than that in perfect flowers with cross-pollination (6.20 ± 0.10 h, $n = 22$; $t = -4.537$, d.f. = 50, $P < 0.001$). When floral closure occurred, 76.67 % of female flowers and 100 % of perfect flowers were fertilized. The average number of fertilized ovules was 2.700 ± 0.445 ($n = 30$) in female flowers and 8.364 ± 0.973 ($n = 22$) in perfect flowers. The difference between morphs was significant ($t = -5.780$, d.f. = 50, $P < 0.001$), due to the difference at the time of floral closure. In this study, pollen grains germinated within 2 h following pollination. The length of time after pollination and the number of fertilized ovules showed a significant quadratic relationship ($r^2 = 0.805$, $F = 237$, $P < 0.0001$; Fig. 2). At the time when floral closure occurred, the number of fertilized ovules was low, and more than half of the ovules were fertilized between 16 and 24 h after pollination (Fig. 2).

The effects of pollen-load size on floral closure and female fitness

For female recipients, 20 grains were adequate to induce closure in over half (63.5 %) of the flowers. PC increased significantly with increasing pollen load (Fig. 3A; $\chi^2 = 23.451$, d.f. = 2, $P < 0.0001$). Pollen-load size had significant positive effects on female fitness (one-way ANOVA, $F_{2,160} = 162.418$, $P < 0.001$ for PD; $F_{2,160} = 14.213$, $P < 0.001$ for NF; $F_{2,160} = 11.486$, $P < 0.001$ for PF; $F_{2,160} = 7.272$, $P = 0.001$ for SN and $F_{2,160} = 5.907$, $P = 0.003$ for SS). NF, PF, SN and SS increased significantly when pollen load was increased from 10 to 20 grains, but there was no significant correlation for an increase from 20 to 30 grains (Fig. 3A).

For perfect recipient flowers, when pollinated with ten pollen grains, no floral closure occurred regardless of

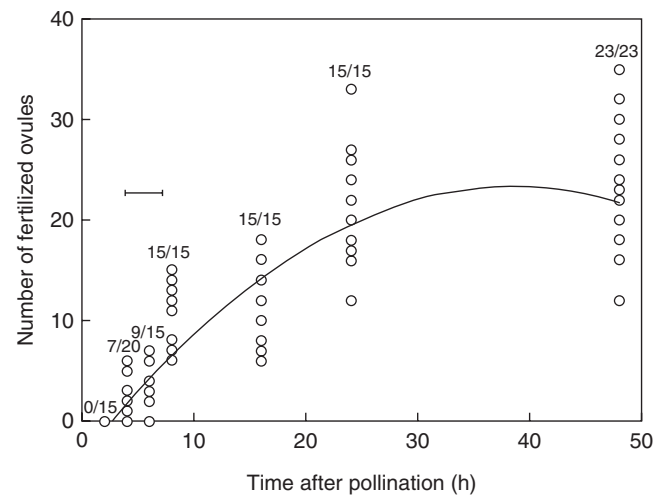


FIG. 2. The relationship between sampling time after pollination and the number of fertilized ovules in *C. delavayi*. Fractions indicate the proportion of fertilized flowers, calculated by number of flowers with fertilized ovules divided by the total number of flowers. The short bar indicates the period during which floral closure occurs.

whether cross- or self-pollen grains were used. Twenty grains induced closure in 26.9 % of flowers that were cross-pollinated and in 36 % of flowers that were self-pollinated. Increasing the pollen load from 20 to 30 grains significantly increased PC in the cross-pollination group (Fig. 3B), but the increase was not significant in the self-pollination group (Fig. 3C). In both cross- and self-pollination groups, PD increased significantly with increasing pollen load as we had expected, but the differences in other indices (NF, PF, SN and SS) were not significant (Fig. 3B, C).

The effects of pollen type on floral closure and female fitness

When 20 grains were received, the proportion of closed flowers was higher in the self-pollination group than in the cross-pollination group (Fig. 4A), but the situation was reversed when 30 grains were received (Fig. 4B). However, the differences between the treatments were not significant ($\chi^2 = 0.488$, d.f. = 1, $P = 0.485$ for 20 grains; $\chi^2 = 1.867$, d.f. = 1, $P = 0.172$ for 30 grains). At any given pollen-load size, there was no significant difference in NF, PF, SN or SS between cross- and self-pollinated flowers (Fig. 4A, B).

The effects of floral morph on floral closure and female fitness

At all three pollen-load sizes, female flowers showed a higher proportion of floral closure than did perfect flowers with cross-pollination ($\chi^2 = 15.261$, d.f. = 1, $P < 0.001$ for 10 grains; $\chi^2 = 9.876$, d.f. = 1, $P = 0.002$ for 20 grains), although this difference was not significant when 30 grains were received ($\chi^2 = 2.468$, d.f. = 1, $P = 0.116$) (Fig. 5A, B). Pollen density was not significantly influenced by floral morph, but female flowers had significantly higher NF, PF, SN and SS than did perfect flowers (with cross-pollination; Fig. 5A, B).

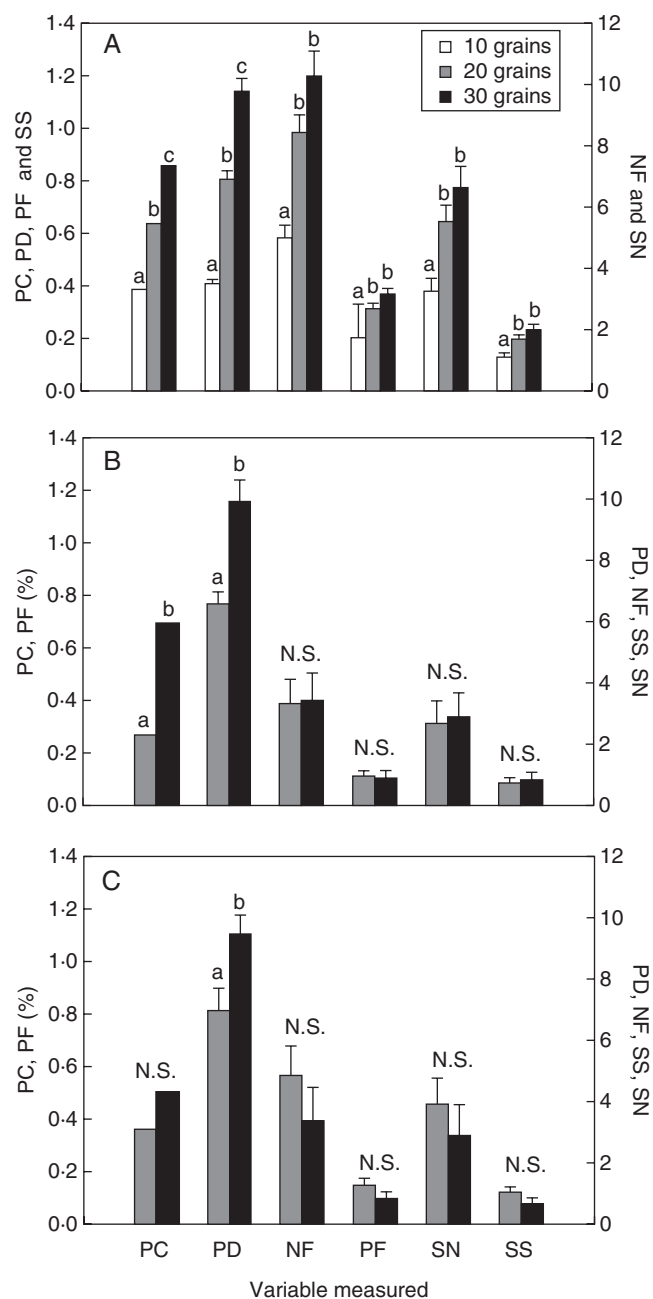


FIG. 3. The effects of pollen-load size (as indicated in the key) on floral closure and female fitness in female recipients (A) and perfect recipient pollinated with cross- (B) or self-pollen (C). Different letters indicate values significantly different at the 0.05 level (N.S., non-significant). PC, Proportion of closed flowers; PD, pollen density (number of pollen grains on a stigma divided by the number of ovules); NF, number of fertilized ovules; PF, proportion of fertilized ovules; SS, seed set per fruit; SN, seed number per fruit. Bars indicate s.e. Note that the data from perfect flowers that received ten grains were not included in the analysis because this number of grains was not sufficient to set fruits – most fruits of these flowers were lost before collection because of wind or rain.

The differences in female fitness between closed and open flowers under the same condition

In general, closed flowers in both female and perfect recipients had higher female fitness than flowers that had not closed

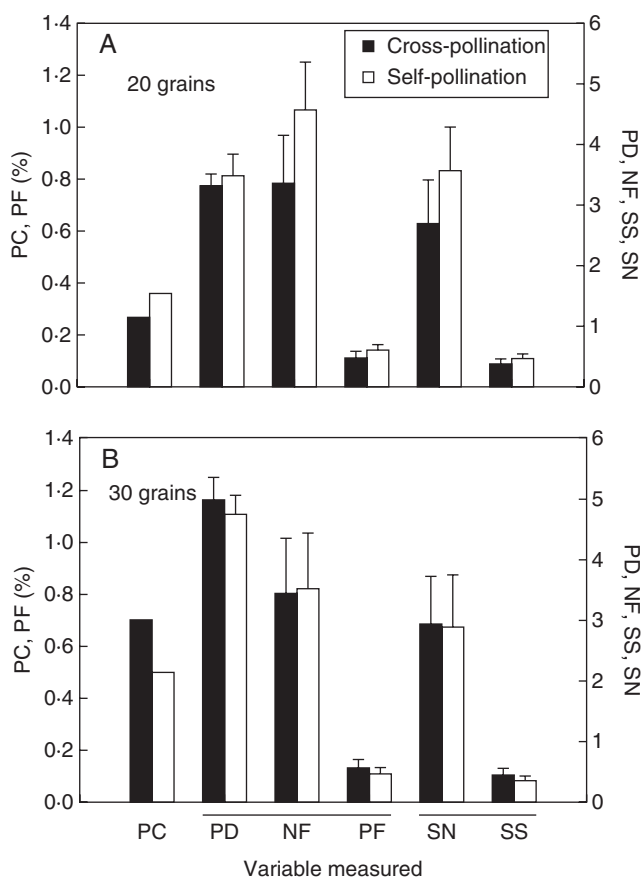


FIG. 4. The effects of pollen type on floral closure and female fitness when (A) 20 and (B) 30 pollen grains were received. There is no significant difference (at the 0.05 level) between cross- and self-pollination in any of these categories. PC, Proportion of closed flowers; PD, pollen density (number of pollen grains on a stigma divided by the number of ovules); NF, number of fertilized ovules; PF, proportion of fertilized ovules; SS, seed set per fruit and SN, seeds number per fruit. Bars indicate the s.e.

under the same condition, but the differences in most of the indices (PD, NF, SN and SS) were not significant across different pollen-load sizes (Table 1). For female recipients, PF was the only index that was significantly different between closed and open flowers across all pollen-load sizes. When the results from all three pollen-load sizes were combined, we found that the lowest PF in closed flowers was still higher than the highest PF in open flowers (Table 1). However, this applied only to female flowers, not to perfect flowers.

When the data were analysed using binary logistic regression, PF was the only index that predicted robustly across all pollen-load sizes whether floral closure would occur in female recipients (Table 2). We failed to find such a predictor for perfect recipients (Table 3).

Fitness consequences of floral closure under natural and manipulated conditions

Under natural pollination conditions, we collected 57 fruits from closed perfect flowers and 40 fruits from closed female flowers. The fruit set among these samples was higher in

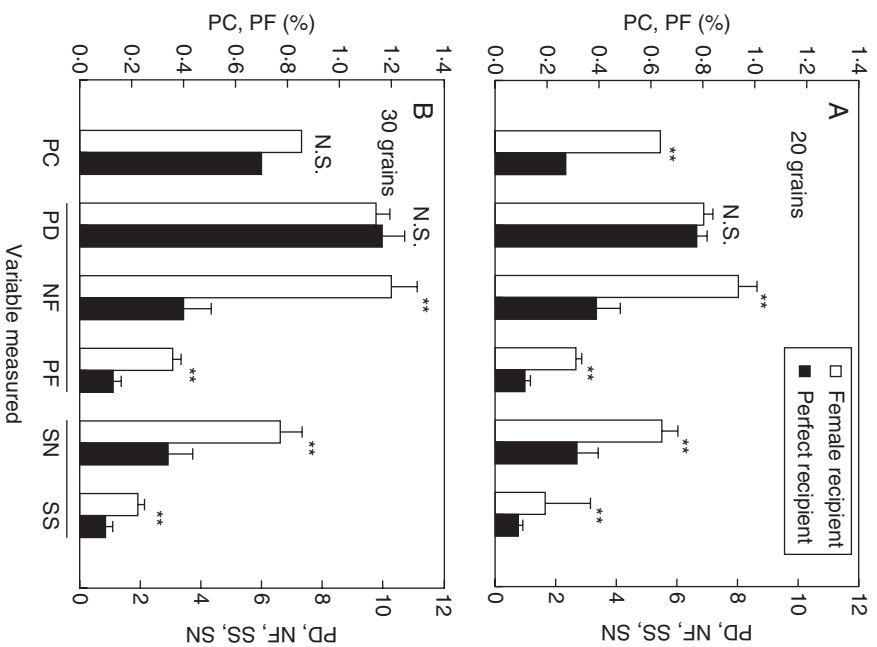


FIG. 5. The effect of floral morph on floral closure and female fitness when (A) 20 and (B) 30 pollen grains were received. ** indicates that there is a significant difference between the female recipient and the perfect recipient at the 0.01 level. PC, Proportion of closed flowers; PD, pollen density (number of pollen grains on a stigma divided by the number of ovules); NF, number of fertilized ovules; PF, proportion of fertilized ovules; SS, seed set per fruit and SN, seeds number per fruit. Bars indicate the s.e.

perfect flowers than in female flowers (0.53 vs. 0.40 , $\chi^2 = 5.89$, d.f. = 1, $P = 0.015$). For fruits with seeds, the seed number per fruit was higher in perfect flowers (12.53 ± 1.45 , $n = 24$) than in female flowers (8.06 ± 1.85 , $n = 16$), but the difference was not significant ($t = 1.857$, d.f. = 44, $P = 0.070$).

All flowers that were hand-pollinated set fruit. Manipulation of flowers to prevent closure did not significantly influence seed production in either perfect or female flowers (for perfect recipients, 17.77 ± 2.03 seeds in manipulated flowers vs. 15.91 ± 1.81 in control, $n = 22$, $t = 0.876$, d.f. = 21, $P = 0.391$; for female recipients, 20.93 ± 1.75 in manipulated flowers vs. 22.33 ± 2.15 in control, $n = 30$, $t = -0.365$, d.f. = 29, $P = 0.531$). However, the difference between morphs was significant, with female flowers showing higher seed production than perfect flowers ($t = 2.177$, d.f. = 50, $P = 0.034$).

DISCUSSION

Time until floral closure

Fertilization was considered to be responsible for floral longevity shortening in some cases (Aizen, 1993; Proctor and

TABLE 1. Comparison of female fitness (mean \pm s.e.) between closed and open flowers at different pollen-load sizes (number of grains on the stigma), for different floral morphs (female and perfect flowers) and pollen types (cross- and self-pollen)

	Female recipients (163)						Perfect recipients with cross-pollination (49)				Perfect recipients with self-pollination (49)			
	10 grains		20 grains		30 grains		20 grains		30 grains		20 grains		30 grains	
	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open
Pollen density	0.42 ± 0.02 (20)	0.40 ± 0.02 (32)	0.85 ± 0.04 (40)	0.72 ± 0.05 (23)	1.12 ± 0.05 (41)	1.21 ± 0.20 (7)	0.78 ± 0.13 (7)	0.77 ± 0.05 (19)	1.13 ± 0.08 (16)	1.23 ± 0.22 (7)	0.81 ± 0.17 (9)	0.81 ± 0.10 (16)	1.02 ± 0.08 (12)	1.19 ± 0.12 (12)
No. of fertilized ovules	5.90 ± 0.64 (20)	4.38 ± 0.59 (32)*	9.10 ± 0.74 (40)	7.26 ± 0.92 (23)	11.05 ± 0.89 (41)	5.57 ± 1.59 (7)	5.8 ± 2.08 (7)	2.42 ± 0.73 (19)	3.44 ± 1.08 (16)	3.43 ± 1.76 (7)	6.44 ± 1.50 (9)	3.50 ± 0.82 (16)	4.33 ± 1.37 (12)	2.67 ± 1.23 (12)
Proportion of fertilized ovules	0.26 ± 0.03 (20)	0.17 ± 0.02 (32)*	0.36 ± 0.03 (40)	0.23 ± 0.03 (23)*	0.39 ± 0.03 (41)	0.20 ± 0.04 (7)*	0.20 ± 0.06 (7)	0.09 ± 0.03 (19)	0.13 ± 0.04 (16)	0.12 ± 0.05 (7)	0.16 ± 0.03 (9)	0.08 ± 0.02 (16)	0.13 ± 0.04 (12)	0.08 ± 0.03 (12)
Seed no. per fruit	3.45 ± 0.69 (20)	3.09 ± 0.52 (32)	5.78 ± 0.67 (40)	5.04 ± 0.88 (23)	6.85 ± 0.78 (41)	5.14 ± 1.68 (7)	5.57 ± 1.95 (7)	1.63 ± 0.56 (19)	3.06 ± 1.04 (16)	2.57 ± 1.29 (7)	5.33 ± 1.22 (9)	2.56 ± 0.78 (16)	3.58 ± 1.24 (12)	2.17 ± 1.20 (12)
Seed set per fruit	0.14 ± 0.03 (20)	0.12 ± 0.02 (32)	0.22 ± 0.02 (40)	0.15 ± 0.02 (23)*	0.24 ± 0.03 (41)	0.18 ± 0.05 (7)	0.18 ± 0.05 (7)	0.05 ± 0.02 (19)*	0.10 ± 0.03 (16)	0.10 ± 0.05 (7)	0.16 ± 0.03 (9)	0.08 ± 0.02 (16)*	0.11 ± 0.03 (12)	0.06 ± 0.03 (12)

Numbers in parenthesis indicate sample sizes.

Asterisks denote values that were significantly different, at the 0.05 level, according to an independent samples *t*-test.

TABLE 2. Binary logistic regression examining the factors affecting floral closure in female flowers, at different pollen-load sizes (number of grains on the stigma)

	10 grains					20 grains					30 grains				
	PD	NF	PF	SN	SS	PD	NF	PF	SN	SS	PD	NF	PF	SN	SS
Chi-square	1.056	3.027	5.393	0.180	0.542	4.124	2.390	10.674	0.457	4.251	0.331	6.316	7.349	0.773	0.682
d.f.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>P</i>	0.304	0.082	0.020	0.671	0.462	0.042	0.122	0.001	0.499	0.039	0.565	0.012	0.007	0.379	0.409
−2 log likelihood	68.237	66.266	63.900	69.113	68.751	78.567	80.301	72.018	82.235	78.441	39.548	33.564	32.531	39.107	39.198

A *P*-value <0.05 indicates that the parameter can robustly predict the occurrence of floral closure.

PD, Pollen density (the number of pollen grains divided by the number of total ovules); NF, number of fertilized ovules; PF, proportion of fertilized ovules; SN, seed number per fruit; SS, seed set per fruit.

TABLE 3. Binary logistic regression examining the factors affecting floral closure in perfect flowers, with cross- or self-pollination, at different pollen-load sizes (number of grains on the stigma)

Predictor	Cross-pollen										Self-pollen									
	20 grains					30 grains					20 grains					30 grains				
	PD	NF	PF	SN	SS	PD	NF	PF	SN	SS	PD	NF	PF	SN	SS	PD	NF	PF	SN	SS
Chi-square	0.007	2.474	3.106	5.537	7.508	0.322	<0.001	0.042	0.084	0.007	<0.001	3.374	2.458	3.698	4.970	1.399	0.877	1.251	0.721	1.587
d.f.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>P</i>	0.934	0.116	0.078	0.019	0.006	0.570	0.996	0.838	0.772	0.934	0.985	0.066	0.117	0.054	0.026	0.237	0.349	0.263	0.396	0.208
−2 log likelihood	30.283	21.961	27.183	24.753	22.782	27.945	28.267	28.225	28.183	28.260	32.671	29.297	30.213	28.973	27.701	31.872	32.394	32.020	32.550	31.684

A *P*-value <0.05 indicates that the parameter can robustly predict the occurrence of floral closure.

PD, Pollen density (the number of pollen grains divided by the number of total ovules); NF, the number of fertilized ovules; PF, the proportion of fertilized ovules; SN, seed number per fruit; SS, seed set per fruit.

Harder, 1995; Ashman and Schoen, 1997; He *et al.*, 2006). However, the timing of these processes has been directly examined only recently, for fireweed (*Chamerion angustifolium*), in which floral closure occurred only 4 h after pollination, well before fertilization (24 h) (Clark and Husband, 2007). Our results demonstrated that floral closure occurred between 4 and 7 h after pollination, at about the same time as the start of fertilization (Fig. 2). Given that fertilized ovules were observed in most ovaries of flowers that had just closed, it is logical to deduce that fertilization events may contribute to the triggering of floral closure in *C. delavayi*. This result appears consistent with the cost–benefit trade-off hypothesis, which suggests that floral longevity should be terminated immediately after reproductive success has been secured, to minimize investment in flower maintenance. Because not all pollen grains loaded onto a stigma fertilized ovules (Table 1 shows that PF is much lower than PD for every combination of floral morph and pollen load), fertilization should be, in comparison with pollen deposition, a much more reliable trigger of floral changes. However, evidence from physiological studies has indicated that fertilization is not essential for post-pollination changes. It has been reported that ethylene, the active signal in most pollination-induced floral changes (Stead, 1992; van Doorn, 1997), is produced at the top of the style, far from the ovary, soon after pollination (Stead and Moore, 1983). In our study, we observed that not all floral closures occurred following fertilization in *C. delavayi*, since 23.33 % of female flowers closed before any ovule had been fertilized. The time lapse between pollination and floral closure in the present study was very similar to the results found in fireweed, but fertilization in *C. delavayi* occurred much earlier than in fireweed (Clark and Husband, 2007). These results allowed us to recognize that we cannot confidently exclude the effects of any pre-zygotic events (such as pollen deposition and pollen tube growth) on floral closure, because it may be masked by the early fertilization in *C. delavayi* (Clark and Husband, 2007). More detailed experiments are required to distinguish between these possibilities.

Factors affecting floral closure

Floral closure in *C. delavayi* was influenced by pollen-load size and floral morph, but not by pollen type. The closure response was sensitive to very small amounts of pollen. For female recipients, 20 grains were sufficient to trigger closing of more than half the flowers. This is fewer than the average number of ovules in a recipient flower, giving a pollen density of <1 (Fig. 3A). To the best of our knowledge, this is the first report of such a sensitive floral closure induced by pollination. Numerous studies have reported a negative effect of pollen quantity on flower longevity. For example, a heavier pollen load led to a shorter morphological female phase in *Campanula americana* (Evanhoe and Galloway, 2002). The time lapse before floral closure in fireweed (*Chamerion angustifolium*) was reduced with increasing pollen load (Clark and Husband, 2007). We obtained a similar result in the present study. The fact that the proportion of closed flowers was significantly increased as the pollen load

increased from 10 to 30 grains (Fig. 3) indicated that pollen-load size was an important factor influencing floral closure.

However, the question remains why some flowers closed after pollination while others, which received the same number of pollen grains, did not. In a study on stigma closure, Fetscher and Kohn (1999) showed that the reopening of stigma lobes after pollination in *Mimulus aurantiacus* appears to be a response to low seed set rather than to low pollen load. However, given that most floral closure in *C. delavayi* occurred shortly after fertilization, much earlier than seed maturity, we believed that fertilization rather than seed production might be the determinant of floral changes. Our results showed that, of all the indices of female fitness, PF was the most reliable predictor of the occurrence of floral closure in female flowers. These results indicated that fertilization (PF) rather than pollen deposition might be a more reliable trigger of floral changes in *C. delavayi*. They also imply that the closure response is influenced not by the absolute female fitness (NF) but by the relative female fitness (PF) (or the residual reproductive ability of a recipient, i.e. $1 - PF$). These findings are consistent with, and contribute additional evidence in support of, the cost–benefit trade-off hypothesis, according to which the termination of floral lifespan should depend on the optimization of female fitness (Primack, 1985; Schoen and Ashman, 1995).

We failed to find any detectable influence of pollen type on floral closure. For a given pollen-load size, self- and cross-pollen induced a similar proportion of closed flowers in *C. delavayi* (Fig. 4A, B), a result similar to the finding that in fireweed the timing of floral closure was only weakly influenced by pollen type (Clark and Husband, 2007). Some studies on stigma behaviour have reported analogous results. For instance, the time of stigma closure was not affected by pollen source (self- or cross-) in the self-incompatible species *Oroxylum indicum* (Sritongchuay *et al.*, 2010). In *Collinsia heterophylla*, for which the timing of stigma receptivity varies depending on pollen identity, even self-pollen was able to fertilize ovules earlier than cross-pollen (Lankinen and Kiboi, 2007). All these results appear inconsistent with the expectations of the cost–benefit hypothesis, which suggests that a flower should remain open until it has received sufficient cross-pollen, in situations where selfing leads to lower female reproductive success. However, we found that ovule fertilization and seed production in *C. delavayi* was not affected by pollen type when pollen load was low (Fig. 4A, B), although this species is partly self-incompatible. Given the fact that, as discussed above, floral change was largely determined by female fitness, this result may explain why we did not detect any effects of pollen type on the occurrence of floral closure.

We examined, for this gynodioecous species, the influence of floral morph on floral closure. Female recipients were more sensitive to pollen deposition than perfect recipients in that they had a higher proportion of closed flowers at any given pollen-load size (Fig. 5A, B). This difference in sensitivity may be partly due to the difference in female fitness between two morphs; this means that, when both morphs received the same number of pollen grains, female flowers had higher female fitness than perfect flowers (Fig. 5A, B). In fact, this fitness difference was also manifested when

pollen deposition was sufficient. In a gynodioecious system, a higher female fitness in female plants is to be expected, and it has been considered an important means of maintaining females in a population (Lewis, 1941; Shykoff *et al.*, 2003; Dufay and Billard, 2012). Another possible explanation for the effect of floral morph on floral closure may be a lower fitness threshold in females, due to their higher susceptibility to pollen limitation. We observed that when floral closure occurred, fewer ovules had been fertilized in female flowers than in perfect flowers (2.7 vs. 8.4). The shorter time until floral closure in female flowers than in perfect flowers (5.38 vs. 6.20 h) may be the consequence of this difference.

Fitness consequences of floral closure and its implications

It has been suggested that floral longevity is plastic and can respond to variations in the rate of fitness accrual (Schoen and Ashman, 1995; Giblin, 2005). For example, low pollen deposition can extend floral longevity in some species (Richardson and Stephenson, 1989; Schoen and Ashman, 1995; Clark and Husband, 2007). This has been proposed as the reason why relatively long floral lifespans have been widely reported in alpine areas, where pollination conditions are considered to be unstable (Bingham and Orthner, 1998; Korner, 2003; Giblin, 2005). In the alpine species used in our study, *C. delavayi*, floral longevity can be prolonged to 7 d or more when pollinators are excluded (Y. Niu and H. Sun, unpubl. res.). However, since floral lifespan ends a few hours after pollen deposition, even at low pollen loads, recipients may receive insufficient pollen to achieve full seed set or fruit set. Over half of the flowers in which closure was induced by natural pollination failed to set fruit, implying that the amount of pollen deposited on the stigma was sufficient to induce floral closure but insufficient to set fruit in many cases. This situation affected female recipients more seriously than perfect recipients, in that they showed significantly lower fruit set in closed flowers, perhaps because female flowers are smaller and less attractive to pollinators (Y. Niu and H. Sun, unpubl. res.), and more sensitive to pollination. Our data also showed that although flowers which have higher PF are more likely to close than the lower ones, there is no significant difference in seed production between the closed and open flowers under the same condition (Table 1). Results from hand-pollination studies indicated that a flower had the potential capability to produce many more seeds (15.91 in perfect flowers and 22.33 in female flowers) if it received sufficient pollen grains. However, a large proportion of ovules could not be fertilized because of early termination of floral lifespan, resulting in low seed production in closed flowers. These results may lead to the interesting conclusion that a flower or plant with an insensitive closure response to pollination or fertilization would have a higher female fitness than a flower or plant with a sensitive closure response because it would receive additional visits from pollinators. We have failed to find a similar report in previous studies on floral changes, but analogous results have been reported in studies on stigma behaviour (Somanathan and Borges, 2001; Richardson, 2004; Milet-Pinheiro *et al.*, 2009; Sritongchuay *et al.*, 2010). For instance, in *Oroxylum indicum*, the permanent stigma closure induced by a low pollen load (300

grains) prevented flowers from receiving enough pollen (900 grains) to set fruit (Sritongchuay *et al.*, 2010). Sensitive stigma closure was also proposed to be responsible for low fruit set in *Heterophragma quadriloculare* (Somanathan and Borges, 2001). Although stigma closure has evident costs, it has been proposed to provide benefits in other respects, such as (1) avoiding or reducing intrafloral self-fertilization (Ritland and Ritland, 1989); (2) reducing interference between male and female functions (Fetscher and Kohn, 1999; Fetscher, 2001); (3) increasing pollen export (Yang *et al.*, 2004); and (4) preventing stigma clogging (Sritongchuay *et al.*, 2010). Floral closure, however, may not provide these benefits in *C. delavayi* for the following reasons. First, since this response occurs, and is more sensitive, in female flowers, the potential benefits related to male function or function interference [i.e. (1), (2) and (3)] can be excluded. Secondly, given that flowers do not receive enough pollen to achieve full seed set, stigma clogging is unlikely to be the case in this study. In some cases, the maintenance of a closed corolla does benefit female fitness. For example, corolla abscission greatly reduced fruit and seed set in *Gentiana straminea* (He *et al.*, 2006). However, in the present study, manipulation to prevent closure did not influence seed production in *C. delavayi* when flowers received sufficient pollen. This implied that the closed corolla *per se* actually provided little benefit for seed production.

Although floral closure was found to be largely determined by the ovule fertilization of the recipient flowers, it is too sensitive to ensure sufficient pollen deposition and leads to evident female fitness costs in *C. delavayi*. These results, taken together with other similar results from studies on stigma closure, may imply that the control of floral receptivity by the recipient flower does not lead to optimal fitness gain. This is a phenomenon that is not easy to explain according to the hypothesis of cost–benefit trade-off, or other hypotheses that consider only the pollen recipient, since they make the assumption that post-pollination phenomena should occur only if reproductive success has been ensured. However, the alternative hypothesis of sexual conflict (Lankinen *et al.*, 2006), which provides an explanation from the perspective of pollen donors, may solve this problem to some extent. It suggests that, by inducing loss of pollen receptivity and preventing additional pollen deposition, the paternity of pollen donors that supply the first pollen to arrive at a flower can be secured under conditions of intense pollen competition (Lankinen *et al.*, 2006). It is thus expected that the pollen amount required to induce floral receptivity loss should be as low as possible, and female fitness may be reduced because insufficient pollen is received. Interestingly, the sensitive floral closure observed in *C. delavayi* and its fitness consequences coincide with these expectations. In fact, the hypotheses based on the perspective of either pollen recipient or pollen donor are not mutually exclusive, and most post-pollination phenomena can be explained according to either of them. However, a cost for female fitness is consistent only with the expectations of the sexual conflict hypothesis. The present work does not provide sufficient evidence to verify the existence of sexual conflict, but the data available allow us to recognize that floral closure in *C. delavayi*, as well as other post-pollination floral changes, are complicated

processes and may not always provide benefits for female fitness.

In conclusion, floral closure in *C. delavayi* occurs after deposition of low pollen loads. It occurs several hours after pollination and may be triggered by fertilization. The closure response is influenced by pollen-load size and floral morph, but not by pollen type. The proportion of fertilized ovules may be the most important factor influencing floral closure. We failed to find any benefits of floral closure for female fitness. Indeed, closure may have deleterious effects on female fitness because of early termination of floral lifespan. We therefore suggest that the control of floral receptivity by the recipient flower does not always lead to optimal fitness gain, since the cost for female fitness implies that the pollen donor may also participate in manipulating floral closure in *C. delavayi*.

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LITERATURE CITED

- Abdala-Roberts L, Parra-Tabla V, Navarro J. 2007. Is floral longevity influenced by reproductive costs and pollination success in *Cohniella ascendens* (Orchidaceae)? *Annals of Botany* **100**: 1367–1371.
- Aizen MA. 1993. Self-pollination shortens flower lifespan in *Portulaca umbraticola* H.B.K. (Portulacaceae). *International Journal of Plant Sciences* **154**: 412–415.
- Ashman TL, Schoen DJ. 1996. Floral longevity: fitness consequences and resource costs. In: Lloyd DG, Barrett SCH. eds. *Floral biology: studies on floral evolution in animal-pollinated plants*. New York, NY: Chapman and Hall, 112–138.
- Ashman TL, Schoen DJ. 1997. The cost of floral longevity in *Clarkia tembloriensis*: an experimental investigation. *Evolutionary Ecology* **11**: 289–300.
- Bernasconi G. 2003. Seed paternity in flowering plants: an evolutionary perspective. *Perspectives in Plant Ecology, Evolution and Systematics* **6**: 149–158.
- Bernasconi G, Ashman TL, Birkhead TR, et al. 2004. Evolutionary ecology of the prezygotic stage. *Science* **303**: 971–975.
- Bingham RA, Orthner AR. 1998. Efficient pollination of alpine plants. *Nature* **391**: 238–239.
- Clark MJ, Husband BC. 2007. Plasticity and timing of flower closure in response to pollination in *Chamerion angustifolium* (Onagraceae). *International Journal of Plant Science* **168**: 619–625.
- Dafni A. 1992. Pollen and stigma biology. In: Dafni A. eds. *Pollination ecology: a practical approach*. Oxford: Oxford University Press, 59–89.
- van Doorn WG. 1997. Effects of pollination on floral attraction and longevity. *Journal of Experimental Botany* **48**: 1615–1622.
- Dufay M, Billard E. 2012. How much better are females? The occurrence of female advantage, its proximal causes and its variation within and among gynodioecious species. *Annals of Botany* **109**, in press. doi:10.1093/aob/mcr062.
- Evanhoe L, Galloway LF. 2002. Floral longevity in *Campanula americana* (Campanulaceae): a comparison of morphological and functional gender phases. *American Journal of Botany* **89**: 587–591.
- Fetscher AE. 2001. Resolution of male–female conflict in an hermaphroditic flower. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **268**: 525–529.
- Fetscher AE, Kohn JR. 1999. Stigma behavior in *Mimulus aurantiacus* (Scrophulariaceae). *American Journal of Botany* **86**: 1130–1135.
- Galen C, Shykoff JA, Plowright RC. 1986. Consequences of stigma receptivity schedules for sexual selection in flowering plants. *American Naturalist* **127**: 462–476.
- Gibbs P, Bianchi M, Taroda R. 2004. Effects of self-, chase and mixed self/cross-pollinations on pistil longevity and fruit set in *Ceiba* species (Bombacaceae) with late-acting self-incompatibility. *Annals of Botany* **94**: 305–310.
- Giblin D. 2005. Variation in floral longevity between populations of *Campanula rotundifolia* (Campanulaceae) in response to fitness accrual rate manipulation. *American Journal of Botany* **92**: 1714–1722.
- Harder LD, Wilson WG. 1994. Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants. *Evolutionary Ecology* **8**: 542–559.
- He YP, Duan YW, Liu JQ, Smith WK. 2006. Floral closure in response to temperature and pollination in *Gentiana straminea* Maxim. (Gentianaceae), an alpine perennial in the Qinghai-Tibetan Plateau. *Plant Systematics and Evolution* **256**: 17–33.
- Hong DY, Ma LM. 1991. Systematics of the genus *Cyananthus* Wall. ex Royle. *Acta Phytotaxonomica Sinica* **29**: 25–51.
- Ishii HS, Sakai S. 2000. Optimal timing of corolla abscission: experimental study on *Erythronium japonicum* (Liliaceae). *Functional Ecology* **14**: 122–128.
- Korner C. 2003. *Alpine plant life*. Berlin: Springer.
- Lankinen A, Kiboi S. 2007. Pollen donor identity affects timing of stigma receptivity in *Collinsia heterophylla* (Plantaginaceae): a sexual conflict during pollen competition? *American Naturalist* **170**: 854–863.
- Lankinen A, Hellriegel B, Bernasconi G. 2006. Sexual conflict over floral receptivity. *Evolution* **60**: 2454–2465.
- Lewis D. 1941. Male sterility in natural populations of hermaphrodite plants: the equilibrium between females and hermaphrodites to be expected with different types of inheritance. *New Phytologist* **40**: 56–63.
- Madjidian JA, Lankinen A. 2009. Sexual conflict and sexually antagonistic coevolution in an annual plant. *Plos ONE* **4**: e5477. doi:10.1371/journal.pone.0005477.
- Milet-Pinheiro P, Carvalho AT, Kevan PG, Schlindwein C. 2009. Permanent stigma closure in Bignoniaceae: mechanism and implications for fruit set in self-incompatible species. *Flora – Morphology, Distribution, Functional Ecology of Plants* **204**: 82–88.
- Paschke M, Abs C, Schmid B. 2002. Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic *Cochlearia bavarica* (Brassicaceae). *American Journal of Botany* **89**: 1250–1259.
- Primack RB. 1985. Longevity of individual flowers. *Annual Review of Ecology and Systematics* **16**: 15–37.
- Proctor HC, Harder LD. 1995. Effect of pollination success on floral longevity in the orchid *Calypso bulbosa* (Orchidaceae). *American Journal of Botany* **82**: 1131–1136.
- Rathcke BJ. 2003. Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *American Journal of Botany* **90**: 1328–1332.
- Richardson SC. 2004. Benefits and costs of floral visitors to *Chilopsis linearis*: pollen deposition and stigma closure. *Oikos* **107**: 363–375.
- Richardson TE, Stephenson AG. 1989. Pollen removal and pollen deposition affect the duration of the staminate and pistillate phases in *Campanula rapunculoides*. *American Journal of Botany* **76**: 532–538.
- Ritland C, Ritland K. 1989. Variation of sex allocation among eight taxa of the *Mimulus guttatus* species complex (Scrophulariaceae). *American Journal of Botany* **76**: 1731–1739.
- Sato H. 2002. The role of autonomous self-pollination in floral longevity in varieties of *Impatiens hypophylla* (Balsaminaceae). *American Journal of Botany* **89**: 263–269.
- Schoen DJ, Ashman TL. 1995. The evolution of floral longevity: resource allocation to maintenance versus construction of repeated parts in modular organisms. *Evolution* **49**: 131–139.
- Shykoff JA, Kolokotronis SO, Collin CL, Lopez-Villavicencio M. 2003. Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. *Oecologia* **135**: 1–9.

- Somanathan H, Borges RM. 2001.** Nocturnal pollination by the carpenter bee *Xylocopa tenuiscapa* (Apidae) and the effect of floral display on fruit set of *Heterophragma quadriloculare* (Bignoniaceae) in India. *Biotropica* **33**: 78–89.
- Sritongchuay T, Bumrung S, Meesawat U, Mazer SJ. 2010.** Stigma closure and re-opening in *Oroxylum indicum* (Bignoniaceae): causes and consequences. *American Journal of Botany* **97**: 136–143.
- Stead AD. 1992.** Pollination-induced flower senescence: a review. *Plant Growth Regulation* **11**: 13–20.
- Stead AD, Moore KG. 1983.** Studies on flower longevity in *Digitalis*: the role of ethylene in corolla abscission. *Planta* **157**: 15–21.
- Webb C, Littleton J. 1987.** Flower longevity and protandry in two species of *Gentiana* (Gentianaceae). *Annals of the Missouri Botanical Garden* **74**: 51–57.
- Yang SX, Yang CF, Zhang T, Wang QF. 2004.** A mechanism facilitates pollination due to stigma behavior in *Campsis radicans* (Bignoniaceae). *Acta Botanica Sinica – English edition* **46**: 1071–1074.