

Wind-Dragged Corolla Enhances Self-Pollination: A New Mechanism of Delayed Self-Pollination

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- **Background and Aims** Delayed self-pollination is a mechanism that allows animal-pollinated plants to outcross while ensuring seed production in the absence of pollinators. This study aims to explore a new mechanism of delayed self-pollination facilitated by wind-driven corolla abscission in *Incarvillea sinensis* var. *sinensis*.
- **Methods** Floral morphology and development, and the process of delayed self-pollination were surveyed. Experiments dealing with pollinator and wind exclusion, pollination manipulations, and pollinator observations were conducted in the field.
- **Key Results** Delayed self-pollination occurs when the abscising corolla driven by wind drags the adherent epipetalous stamens, thus leading to contact of anthers with stigma in late anthesis. There is no dichogamy and self-incompatibility in this species. The significantly higher proportion of abscised corolla under natural conditions as compared with that in wind-excluding tents indicates the importance of wind in corolla abscission. When pollinators were excluded, corolla abscission significantly increased the number of pollen grains deposited on the stigma and, as a result, the fruit and seed set. Only half of the flowers in plots were visited by pollinators, and the fruit set of emasculated flowers was significantly lower than that of untreated flowers in open pollination. This species has a sensitive stigma, and its two open stigmatic lobes closed soon after being touched by a pollinator, but always reopened if no or only little pollen was deposited.
- **Conclusions** This delayed self-pollination, which involved the movement of floral parts, the active participation of the wind and sensitive stigma, is quite different from that reported previously. This mechanism provides reproductive assurance for this species. The sensitive stigma contributes to ensuring seed production and reducing the interference of selfing with outcrossing. The pollination pattern, which combines actions by bees with indirect participation by wind, is also a new addition to ambophily.

Key words: Ambophily, anther movement, Bignoniaceae, corolla abscission, delayed self-pollination, *Incarvillea sinensis* var. *sinensis*, reproductive assurance, stigma closure.

INTRODUCTION

The reproductive assurance hypothesis is often used to explain why self-pollination has evolved repeatedly in plants (reviewed by Barrett, 1988; Wyatt, 1988). Selection for reproductive assurance is regarded as one of the major factors involved in the evolutionary transformation from animal pollination to self-pollination (Stebbins, 1957). When a scarcity of pollinators and/or mates strongly limits reproduction, self-pollination can be achieved as a means of ensuring seed production in some self-compatible species (Lloyd and Schoen, 1992). The modes of self-pollination include cleistogamy, geitonogamy, facilitated self-pollination and autonomous self-pollination (Lloyd and Schoen, 1992). However, only autonomous self-pollination provides reproductive assurance because it can be autonomously achieved by plants when pollinators are absent or present in low numbers (Lloyd, 1979, 1992). Lloyd and Schoen (1992) classified this autonomous

self-pollination mode into three general categories, termed 'prior', 'competing' and 'delayed', according to whether self-pollination occurs before, during or after opportunities for outcrossing in a flower, respectively. Among these modes, only delayed autonomous selfing incurs no pollen and/or seed discounting costs at the flower level (Schoen and Brown, 1991; Lloyd, 1992). Therefore, delayed autonomous selfing is generally regarded as adaptive because it ensures seed production when pollinators are scarce, yet allows outcrossing to predominate when they are abundant (Wyatt, 1983; Cruden and Lyon, 1989; Fenster and Martén-Rodríguez, 2007).

Despite the selective advantage of delayed autonomous self-pollination, surprisingly few mechanisms are involved. Delayed autonomous self-pollination can be achieved through changes to the spatial arrangement of male or female organs within the flower leading to pollen–stigma contacts at the end of anthesis (Faegri and van der Pijl, 1979; Lloyd and Schoen, 1992; Rathcke and Real, 1993; Klips and Snow, 1997; Eckert and Schaeffer, 1998; Kalisz *et al.*, 1999; Liu *et al.*, 2006). Corolla movements

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or petal wilting can also contribute to delayed self-pollination (Dole, 1990, 1992; Sun *et al.*, 2005). Both mechanisms occur actively without involving any external vectors.

Besides autonomous self-pollination, wind pollination could also be viewed as a potential alternative solution, providing reproduction assurance in the case of low pollinator abundance. A number of studies suggest that the combination of wind and insect pollination occurs in entomophilous plants (Vroege and Stelleman, 1990; Norman *et al.*, 1997; Mahy *et al.*, 1998; Goodwillie, 1999). The hypothesis that wind pollination may provide reproductive assurance was directly supported by studies on two primarily insect-pollinated plants, in which wind pollination was found in their colonized areas with low insect abundance (Berry and Calvo, 1989; Gómez and Zamora, 1996). In both cases, the pollen was directly transported to the stigma by air flow. Dafni and Dukas (1986) reported that in an insect-pollinated plant species the pollen was dispersed through wind vibrating flowering stems, providing reproductive assurance in an unfavourable season when insects were scarce.

The present study provides evidence that wind is indirectly involved in delayed self-pollination through propping corolla movements in *Incarvillea sinensis* var. *sinensis*. Preliminary field observations indicated that the anthers of didynamous stamens in this species were positioned under the broad lobes of the stigma and clung to the style before anthesis initiation. The corollas are easily blown off by the wind in late anthesis and, by this process, the epipetalous stamens can be dragged, slipping forward and past the stigma. In addition, although the showy, nectar-rich flowers appear to be adapted for animal pollination, they receive only a few animal visits during the peak blooming period. However, the fruit set was high under natural conditions. It was therefore hypothesized that self-pollination without participation of pollinators might contribute to the high fruit and seed production and, in addition, that the wind might play an important role in the movement of corolla and stamens leading to pollen–stigma contact. The aim of this study was to test this prediction through addressing the following questions. (1) Does delayed self-pollination occur in this species? And what floral morphological mechanisms are involved? (2) Is the wind involved in the self-pollination? And how? (3) What extent does the delayed self-pollination contribute to fruit and seed production? And can it provide reproductive assurance for this species?

MATERIALS AND METHODS

Study species and sites

Incarvillea sinensis Lamarck var. *sinensis* (Bignoniaceae) is an annual herb endemic to China, growing mainly on open hillsides and fields (Zhang and Santisuk, 2003). It is 20–80 cm tall, with a sparse raceme that produces 4–60 purple to red, campanulate/funnel-form flowers. These flowers are bisexual (perfect) and are 2.5–4.0 cm in diameter. A single flower can secrete $1.40 \pm 0.34 \mu\text{L}$ nectar

with $24.90 \pm 2.81\%$ sucrose during anthesis (R. Qu *et al.*, unpubl. res.). The androecium is didynamous and inserted at the base of the corolla tube. The stigma is composed of two round lobes covered with dense short hairs (Zhang and Santisuk, 2003), and the stigmatic lobes are sensitive to mechanical or pollinator touch (Yang, 2005). Flowering occurs from early July to late August, peaking in late July in the study region (R. Qu *et al.*, unpubl. res.).

The study was conducted from July to September, 2005 and 2006 in Mu Us Sand land (39°02'N, 109°51'E), in the south-east of the Ordos Plateau in Inner Mongolia, China. This area is a semi-arid desert with a mean annual precipitation of 260–450 mm and a mean annual temperature ranging from 7.5 to 9.0 °C. Over 200 d per year have a wind speed of more than 5 m s^{-1} (Geography Department of Peking University, 1983; Zhang, 1994). The landscape is characterized by fixed, semi-fixed and moving sand dunes, and patchy vegetation dominated by psammophytic shrubs and herbs such as *Hedysarum leave* or *Psammochloa villosa* (Zhang, 1994). Five patches of *I. sinensis* var. *sinensis* were selected at random in this region. These patches are about 100–500 m away from each other.

Flowering features

In each of the selected five patches, 20 individuals were randomly selected and marked prior to blooming. The phenological development of each single flower on each marked plant was recorded every 30 min throughout each day, and nocturnal observations, including changes of the angle between the long axis of the flower bud and the rachis during floral development, were made. The time of anther dehiscence, the time of the opening and abscising of the corolla were also noted. In addition, the number of flowers that opened on each plant per day was also counted every morning.

Floral morphological development and changes during anthesis

Three small flower buds, each from 25 individuals (75 flower buds in total), were tagged. The floral morphology and the movements of floral parts were then examined at 2-h intervals from young flower bud to corolla abscission. Particular attention was paid to the positional changes of the stamens and pistil, the movement of the stigmatic lobes and the movement of the corolla. To estimate the percentage of corolla abscission under natural conditions, six $2 \times 2\text{-m}$ plots that contained more than 50 plants were randomly selected. During the peak blooming period, the number of flowers opening in each plot was counted at 0700 h every morning, and the number of abscised corollas was recorded every 30 min from 0800 to 1130 h every morning for seven consecutive days. Wind speed was measured using a portable anemometer (AVM-01, TES Electrical Electronic Corp., Taiwan) when most corollas abscised in the plots. To examine the possibility that corollas were blown off by the wind during the whole flowering period, climate data for each day, including wind speed and precipitation, were collected every hour



FIG. 1. The process of floral development and delayed self-pollination. (A–E) The growth dynamics of stamens and pistil in the process of flower development, showing the process of lift of anther sacs, anther adherence and the formation of a clip-like structure [A–C, the early stage to middle stage of flower bud growth (the view distal to rachis); D, E, the abdominal (distal to the rachis) and dorsal (near the rachis) view of the clip-like structure comprising stamens and pistil in the opening flower]; SL, stigmatic lobes; PR, flat oblong projection on the front connective; PA, large papillae at the back of connective; ST, style; AS, anther sac; SF, staminal filament; GC, globose connective. (F, G) Corolla detachment from its floral receptacle and corolla slipping forward along the style during corolla abscission driven by wind. (H) The wilted corolla on the flower in wind-excluding tents. (I, J) Stamen movement and self-pollination process when the corolla is abscised, showing the upward turning of anthers due to the blockage of the lower stigmatic lobe, and self-pollen deposition on stigma. (K) The closed stigmatic lobes after being pollinated by bees.

from a weather station in the study site from July to September, 2006. The hourly maximal wind speed from 0800 to 1200 h per day was then picked out and averaged, and the mean maximal wind speed between 0800 and 1200 h every day from July to September was plotted (see Fig. 2A). In addition, in order to examine whether similar floral structure and the movement of floral parts occur commonly in the field, the samples were expanded to 15 randomly selected patches and the floral features of about 160 flowers on 100 individuals in each patch (2599 flowers from 1500 individuals in total) were investigated over a period of 2 years.

To examine the role of the corolla abscission in inducing self-pollination, half of the corolla was cut off longitudinally in 20 opening flowers that had not been visited by insects, the remaining half corolla was then dragged manually along the length of the style with a pair of fine tweezers simulating the process of corolla abscission; the movement of the floral parts in the process of manipulation were then

photographed (Fig. 1I–J). Meanwhile, it was checked whether any pollen remaining in the anthers could be deposited on the stigma of the same flower during the manual movement of the corolla.

To confirm the behaviour of the stigma and to determine the relationship between stigma behaviour and self-pollination, three groups of flower buds (100 flower buds per group) were labelled in pollinator-excluding tents. These tents were 1.5 × 1.5 × 1.0-m cuboids constructed of white nylon tent-window fabric attached to a woody frame that was placed over plants. These labelled flower buds were emasculated with a pair of forceps 1 d before anthesis initiation. After anthesis initiation, the surface of the open stigmatic lobes of the three groups of flowers were respectively treated as follows: (1) brushed with the back of one dead bee without any pollen on its body, providing only mechanical stimulation; (2) brushed with one dead bee but with many pollen grains on its back, simulating the touch of a pollen-laden pollinator; and (3) sprinkled

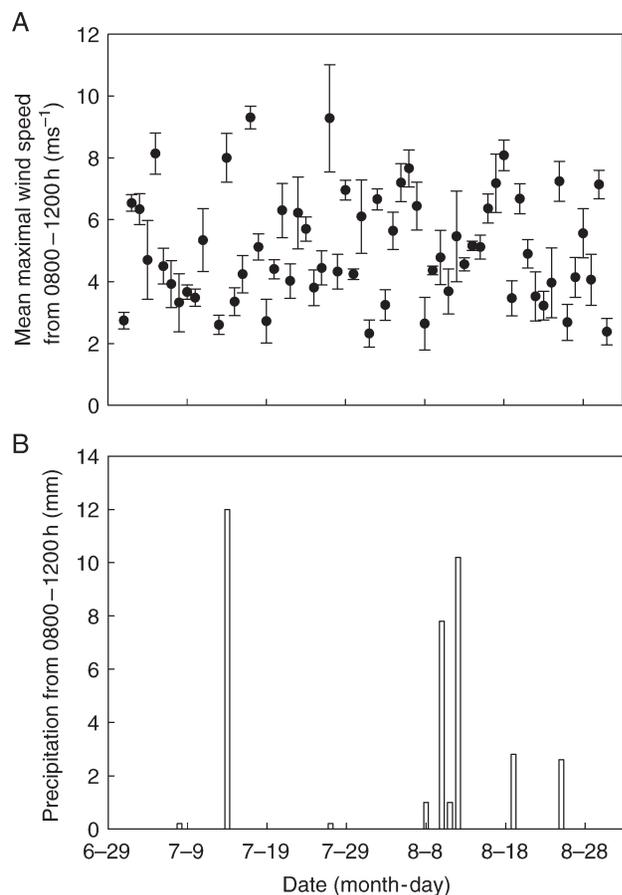


FIG. 2. (A) The daily mean maximal wind speed during 0800–1200 h from July to September, 2006. (B) Daily precipitation during 0800–1200 h from July to September, 2006.

with pollen grains but with no touch, providing only pollen stimulation. The behaviour of stigmatic lobes in each tagged flower was then examined every 5 min after treatment.

Capability for self-fertilization

We first tested pollen viability and stigma receptivity, and assessed P/O ratio (pollen–ovule ratio) to determine the possibility of self-fertilisation in *I. sinensis* var. *sinensis*. In total, 150 flower buds about to open were selected and labelled from 75 individuals enclosed in pollinator-excluding tents. Fifteen buds or flowers were collected at 1-h intervals after anther dehiscence (until the corolla abscission), and the viability of pollen was estimated as the percentage of pollen grains stained by TTC is a well known method (1.0% by weight in 10% sucrose) from over 300 pollen grains (Mulugeta *et al.*, 1994). Meanwhile, the stigma receptivity of collected flowers was tested with the benzidine/ H_2O_2 method described by Dafni *et al.* (2005).

To estimate the P/O ratio, 30 flower buds about to open were collected from 15 individuals at random. One mature but undehiscent anther from a long stamen and a short stamen in each flower bud was respectively removed. The pollen grains were then counted under an

Olympus light microscope ($60\times$) from a ‘squash’ preparation following initial softening of the tissues in 3 mol L^{-1} HCl. All ovules in every ovary from the 30 flowers were also counted, using the same method.

Secondly, pollinator exclusion combined with hand-pollinations was used to quantify the ability of this species for self-fertilization. Hand-pollination experiments were carried out in two patches between 20 and 30 July, 2006. In the pollinator-excluding tents, four treatments were performed following the method described by Dafni (1992): (1) automatic self-pollination – the flowers were not manipulated; (2) apomixis – buds were emasculated, no hand-pollination; (3) hand self-pollination – buds were not emasculated, hand-pollination with the pollen from the same flower; (4) hand cross-pollination – buds were emasculated, hand-pollination with pollen from other plants at least 50 m away. At least ten groups of flowers (20 flowers per group) were treated in each treatment. The fruit set of these flowers was counted in each treatment after 7 d of flower wilting. One month later, 30 fully mature fruits per treatment were collected in each treatment, and the seed set (ratio of seeds to ovules) per fruit was measured. The fruit and seed set of ten groups of flowers (60 flowers per group) were also measured under natural conditions in five selected patches.

Self-pollen deposition

To examine whether corolla abscission can increase the number of pollen grains deposited on stigma and simultaneously decrease the number of pollen grains remaining in anthers, 30 individuals were enclosed using pollinator-excluding tents and two flowers on each individual (60 flowers in total) were tagged. The two flowers on each plant were respectively removed before and after corolla abscission. The stigma of each flower was carefully excised and mounted in a drop of Alexander’s staining liquid (modified from Alexander by Dafni, 1992) on a slide. The pollen grains on the stigmatic lobes were counted under an Olympus light microscope ($60\times$). The difference in the number of pollen grains on the stigmatic lobes before and after corolla abscission could be considered as the contribution of corolla abscission to self-pollen deposition. Similarly, the anthers of two long and two short stamens in each flower were removed before and after corolla abscission, respectively, and mounted in a drop of 2 mol L^{-1} HCl solution for 3 h on a slide. Subsequently, the pollen grains were counted under a light microscope ($60\times$). The difference in the number of pollen grains that remained in the anthers before and after corolla abscission was considered to indicate the contribution of corolla abscission to pollen shedding.

Wind-exclusion experiments

To estimate the importance of the wind for corolla abscission, randomly six 2×2 -m plots containing >50 plants were selected and then enclosed with wind-excluding tents which were constructed of white nylon fabric attached to a wooden frame. On five consecutive days, the number of

flowers opening in each tent was counted in the morning, and the number of abscised corollas was recorded at the end of anthesis (1200 h). The proportion of flowers with abscised corolla was then calculated and compared with that in the six observed plots that were open to the wind.

To determine whether the movement of floral parts and corolla abscission driven by wind actually increased fruit and seed set, ten groups of flower buds (60 flower buds per groups) were enclosed using wind-excluding tents to prevent corolla abscission and pollinator visitation. In addition, another ten groups of flower buds (60 flower buds per groups) were also enclosed using pollinator-excluding tents to permit wind entry and corolla abscission but to prevent pollinator visitation. These tents were removed after fruiting. The fruit and seed set were counted and compared between the two treatments.

Pollinator observations and estimation of the contribution of self-pollination to reproduction

Observations of flower visitors were made in three out of five selected patches between 0100 and 1200 h from 20 July to 10 August (120 h in total). The visitors, their behaviours and visitation frequency were noted in two 2×2 -m plots every 30 min from the first flower blooming until almost all corollas fell off during the following 7 d. The flowers visited by pollinators were marked and the percentage of flowers visited by pollinators in the two plots was calculated daily. Insects were caught by net and killed, using ethyl acetate.

In addition, to estimate the contribution of self-pollination to fruit and seed production, two treatments were used. (1) Measurement of fruit and seed set of the flowers that were emasculated and exposed to pollinator: ten groups of flower buds (60 flower buds per groups) were selected randomly from five patches. These flower buds were then emasculated before anther dehiscence and exposed to pollinators under natural conditions. The fruit and seed set of each group of flowers were then checked to assess the contribution of animal pollination to reproduction. (2) Fruit and seed set of ten groups of untreated flowers (60 flowers per groups) were checked under natural conditions. The contribution of self-pollination to reproduction was estimated by subtracting the fruit and seed set of emasculated flowers from that of untreated flowers.

Statistical analysis

All data were examined for normal distribution with a one-sample Kolmogorov–Smirnov test. Variables not normally distributed were log-transformed to meet assumptions of normality. One-way ANOVA was used to identify any significant difference in fruit and seed set among various pollination treatments. Independent-sample *t*-tests were respectively used to identify the significant difference in the proportion of abscised corollas between the wind-exclusion tents and open plots, the difference in the fruit and seed sets between the flowers in the wind-excluding tents and those whose corollas abscised naturally, and the

difference in the fruit set between the emasculated flowers and the untreated flowers. A paired-samples *t*-test was used to identify the significant difference in the number of pollen grains deposited on the stigma and remaining in the anthers between before and after corolla abscission. All analyses were performed in SPSS 13.0 for Windows (SPSS, 2004).

RESULTS

Flowering features

The flowers were spirally and sparsely arranged on the raceme. The angle between the long axis of the flower bud and the rachis was small ($37.00 \pm 2.26^\circ$, $n = 75$ flowers) at the young flower bud stage. It gradually increased with growth of the bud, and reached $84.10 \pm 2.99^\circ$ ($n = 75$ flowers) extending almost horizontally when the flower opened. The flowers opened nocturnally, between 0230 and 0430 h. The duration of one flower lasted 5–9 h ($n = 75$ flowers). One plant opened 1–8 flowers per day.

Floral features and developments

In the flowers of *I. sinensis* var. *sinensis*, each anther is composed of two separate anther sacs, connected by a globose connective terminating each staminal filament (Fig. 1C). A flat, oblong projection (0.59 ± 0.05 mm high, 0.75 ± 0.04 mm wide, $n = 75$ flowers) is attached at the front of the connective (distal from the rachis) (Fig. 1A, B). The top of the projection secretes some mucilage during the growth of the flower bud. A large papilla is joined to the back of the connective (near the rachis; Fig. 1B, E). The style is appressed to the upper side of the corolla tube (Fig. 1D, K). In the process of flower bud growth, two parallel sacs of the anther splay out at the connective (Fig. 1B), and one of them then lifts upward and lines up with the other before anthesis initiation (Fig. 1C–E); at the same time, the straight staminal filament spontaneously bends slightly towards the style (Fig. 1B, C), and the two anthers of long and short stamens, respectively, adhere together in the front of the style through their flat, oblong projections on the connective (Fig. 1C, D). Finally, two pairs of adherent anthers become parallel adnate to the style below the stigmatic lobes (Fig. 1D, E), and their large papillae joined to the back of the connectives are positioned at the sides of the style (Fig. 1E). The adherent flat, oblong projections and two papillae form a clip-like structure enclosing the style. This clip-like structure ensures that the anthers can slip along the style. The anthers dehiscence longitudinally about 2–4 h before anthesis initiation. Two stigmatic lobes opened and curled downwards but make no contact with the anthers of the long stamens before anthesis initiation (Fig. 1D, E).

The movements of corolla and stigmatic lobes

During late anthesis (0930–1130 h) corollas began to abscise when the wind speed was over 2 m s^{-1} , the

opened and unwilted corollas slipping forwards and falling off (Fig. 1F, G). When the wind speed was low ($<2 \text{ m s}^{-1}$), the corollas did not move and abscise, and wilted on the floral receptacle (Fig. 1H). In the six plots (of $2 \times 2 \text{ m}$) under natural conditions, $95.13 \pm 0.75 \%$ of the corollas abscised and fell off by 1130 h on seven consecutive observation days. Thus, there were almost no opening flowers in the afternoon. At the study site, from early July to the end of August, 2006, the mean daily maximal wind speed from 0800 to 1200 h was more than 2 m s^{-1} (Fig. 2A). Moreover, rainfall seldom occurred during this period (Fig. 2B). Strong winds and rare rainfall made it possible for corollas to be blown off.

When the corolla moved forwards driven by wind, the anthers that are inserted at the base of the corolla were dragged by the corolla and slipped along the style under the limits imposed by the clip-like structure of the anthers (Fig. 1I). When the anthers reached the stigma they turned upward, being blocked by the opening stigma lobes, and the filaments bent severely under the pressure of the moving corolla. Finally, the anthers were forcibly dragged to pass the stigma by the continued moving corolla. After the anthers passed the stigma, their dehiscence faces touched the inner surfaces of two stigmatic lobes, and brushed pollen onto them (Fig. 1J). Meanwhile, with the help of the force released from the severely bent filaments after the anthers had passed the stigma, the dehisced sacs were able to shed pollen grains onto the inner surface of the stigmatic lobe. In 15 patches investigated, $96.37 \pm 1.35 \%$ flowers consistently showed the same floral structure and the process of movement by the floral parts. However, if the flower had been visited and pollinated by bees, the two open stigmatic lobes would close upon contact from each other and the surface of the stigma would be covered (Fig. 1K). The closed stigmatic lobes failed to block the anthers and the pollens could not be deposited onto the surface of the stigma. Therefore, self-pollination could not be achieved.

If they were not touched, the stigmatic lobes remained open until corolla abscission. After pollinator visitation, two open stigmatic lobes always closed tightly in the flowers observed. Of the flowers treated by manual stimulation, 92.74% of the stigmatic lobes brushed by the back of bees without pollen grains closed after 2–10 s of treatment, but reopened 15–25 min after closure ($n = 100$ flowers); all the stigmatic lobes brushed by the back of bees with many pollen grains closed permanently ($n = 100$ flowers); those stigmatic lobes that were only sprinkled with pollen grains but not brushed by the bee's body did not close at all ($n = 100$ flowers). The closure of stigmatic lobes could not be caused by the movement of floral parts and contact by pollen during the process of corolla abscission because the inner surface of the stigmatic lobes had not been substantially stimulated by them.

Capability for self-fertilisation

Staining with TTC showed that the proportion of viable pollen was $63.02 \pm 1.15 \%$ ($n = 15$ flowers) when the anthers dehisced, and peaked ($71.98 \pm 1.23 \%$) about

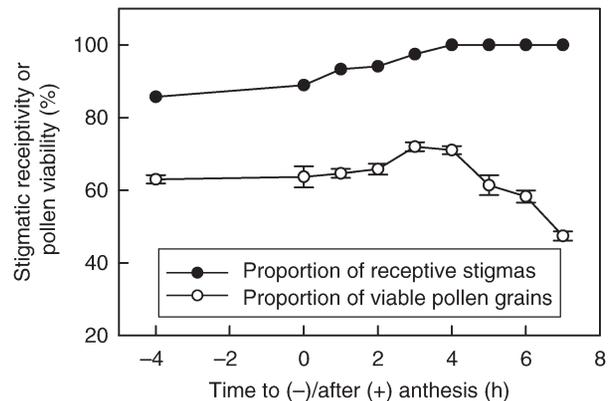


FIG. 3. The dynamics of the proportion of viable pollen grains and receptive stigmas over time after anther dehiscence.

3–4 h after anthesis initiation. Subsequently, the proportion of viable pollen decreased rapidly, reaching $47.41 \pm 1.25 \%$ when the corollas abscised (6–9 h after anthesis initiation) (Fig. 3). The proportion of receptive stigmas was 85.71% ($n = 15$ flowers) when the anther dehisced. This reached 100% 3–4 h after anthesis initiation and remained at 100% until corolla abscission (Fig. 3). This indicated that no dichogamy occurred in this species.

There were 180.80 ± 5.30 ovules and $18\,816.27 \pm 326.70$ pollen grains in one flower ($n = 30$). The P/O ratio was 104.7.

Both fruit and seed sets followed by automatic self-pollination, hand self-pollination and hand cross-pollination showed no significant difference (fruit set: $F = 0.04$, d.f. = 2,27, $P = 0.96$; seed set: $F = 0.48$, d.f. = 2,87, $P = 0.62$; Fig. 4). The emasculated and caged flowers did not develop into fruit, indicating that no apogamy had occurred in this species. The natural fruit set ($85.93 \pm 1.63 \%$, $n = 10$ groups of flowers) was significantly lower than that for hand self- and cross-pollination ($t = -5.77$, d.f. = 18, $P < 0.001$; $t = -7.23$, d.f. = 18, $P < 0.001$, respectively).

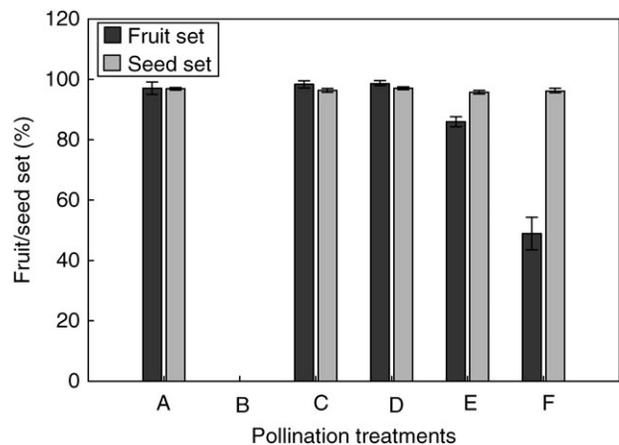


FIG. 4. Comparison of fruit and seed set among pollination treatments. (A) Automatic self-pollination. (B) Apomixis. (C) Hand self-pollination. (D) Hand cross-pollination. (E) Natural pollination. (F) Emasculatation and exposure to pollinators.

However, the seed set under natural conditions showed no significant difference from that of automatic self-pollination, hand self-pollination or hand cross-pollination ($F = 1.38$, d.f. = 3,116, $P = 0.26$).

Self-pollen deposition

Because the anthers are appressed to the upper part of the corolla tube and are covered by the lower stigmatic lobe, after the anthers dehisced, some pollen grains fell onto the edge of the lower stigmatic lobe. Before corolla abscission, the number of pollen grains deposited on the stigma via this route reached 66.9 ± 6.13 (ranging from seven to 135; $n = 30$ flowers). This indicated that autonomous self-pollination occurred before corolla abscission.

The pollinator-exclusion experiments revealed that the number of pollen grains on the stigma increased almost five-fold after corolla abscission, i.e. from 66.90 ± 6.13 before to 337.07 ± 26.37 after corolla abscission ($t = -11.27$, d.f. = 29, $P < 0.001$). At the same time, the total number of pollen grains remaining in the anthers per flower decreased significantly, from 6574.33 ± 274.28 to 1049.20 ± 103.80 ($t = 17.28$, d.f. = 29, $P < 0.001$). Compared with that before corolla abscission, the number of pollen grains remaining in the anthers of the long and short stamens decreased by 81.80 and 86.45 %, respectively, after corolla abscission. Observations indicated that the manual moving corolla simulating corolla abscission driven by the wind in fact enhanced the self-pollen deposition on the stigma.

Wind-exclusion experiments

The proportion of abscised corollas was significantly lower in the wind-exclusion tents (only 1.44 ± 0.13 %) than that in open plots (93.13 ± 0.75 %) ($t = 74.13$, d.f. = 58, $P < 0.001$).

When pollinators were excluded, the fruit and seed sets of flowers in the wind-excluding tents were both significantly lower than those whose corollas abscised naturally (fruit set: 64.28 ± 3.79 and 98.75 ± 2.25 %, respectively; $t = 7.15$, d.f. = 18, $P < 0.001$; seed set: 41.32 ± 4.17 and 96.87 ± 3.24 %, respectively; $t = 12.35$, d.f. = 18, $P < 0.001$; Fig. 5). This indicated that self-pollination facilitated by corolla abscission increased fruit set by over 30 % and seed set by over 50 %.

Pollinator observations and estimation of the contribution of self-pollination to reproduction

Only *Bombus sporadicus* and *Anthophora borealis* pollinated *I. sinensis* var. *sinensis*. *B. sporadicus* appeared mainly from 0630 to 0900 h (3–6 h after anthesis initiation), while *A. borealis* appeared mainly from 0800 to 1100 h (5–8 h after anthesis initiation). They consecutively visited 1–5 flowers on the same individual in one foraging bout. The time spent by one pollinator on a single flower was 2.55 ± 0.49 s ($n = 30$) by *B. sporadicus*, and 1.05 ± 0.12 s ($n = 30$) by *A. borealis*.

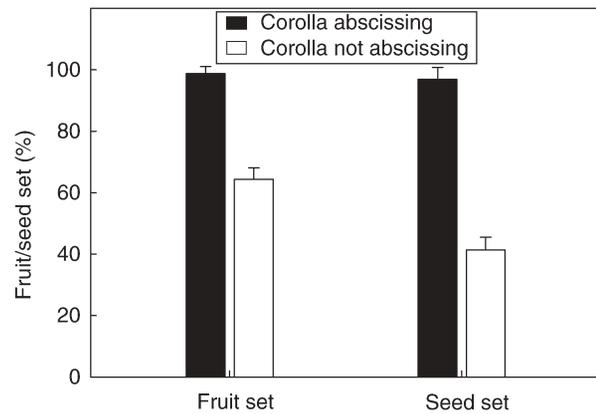


FIG. 5. Comparison of fruit and seed set between flowers whose corollas abscised and those whose corollas were prevented from abscising.

During more than 120 h of observations in the flowering season, only 5.25 ± 1.16 visits were recorded by the two bee species per day in one 2×2 -m plot ($n = 7$ d). According to these observations only 49.96 ± 7.86 % ($n = 14$) of the flowers that opened in plots were visited. When being exposed to open pollination, the fruit set of the emasculated flowers (48.90 ± 5.37 %) was significantly lower than that of the untreated flowers (85.93 ± 1.63 %) ($t = -6.94$, d.f. = 18, $P < 0.001$; Fig. 4). The remaining fruit set of approx. 37 % was undoubtedly the result of self-pollination.

DISCUSSION

Mating system

Self-incompatibility has been reported in 17 species of Bignoniaceae (reviewed in Gibbs and Bianchi, 1999). However, the present hand-pollination experiments revealed that *I. sinensis* var. *sinensis* is a completely self-compatible species. The P/O ratio of *I. sinensis* var. *sinensis* (104) is also within the range that Cruden (1977) assigned to 'obligate autogamy' ($4.7 \pm 0.7 < P/O < 168.5 \pm 22.1$ in this class).

Delayed self-pollination and reproductive assurance

The pattern of floral development in *I. sinensis* var. *sinensis*, including the bent growth of filaments, lift of anther sacs and adherence of anthers in pairs, is unusual in flowering plants (Angiosperms). The clip-like structure, consisting of two adherent connectives, ensures that the anthers can slip rigidly along the style and brush pollen onto the stigmatic lobes when the abscising corolla drags stamens. This floral structure is quite different from that of *Mimulus guttatus*, the only reported case of delayed self-pollination facilitated by corolla abscission, in which the four stamens are completely separated and do not cling to the pistil (Dole, 1990, 1992). Therefore, the floral structure of *I. sinensis* var. *sinensis* can be regarded as a specialization for self-pollination facilitated by corolla-dragging anther movement.

The present study reveals that the stigmas of *I. sinensis* var. *sinensis* are receptive and the pollen grains are viable when the corolla abscised, and that no dichogamy occurs. Corolla abscission in late anthesis significantly increased the number of pollen grains deposited on the stigma, indicating that the corolla abscission resulted in self-pollination within flowers. When pollinators were excluded, the fruit and seed set in those flowers whose corollas were prevented from abscising were significantly lower than those whose corollas were allowed to abscise naturally. This indicated that the corolla abscission actually enhanced the seed production sired by selfing. Autonomous self-pollination can also occur before corolla abscission, but this process deposited only 66.9 ± 6.13 pollen grains on the stigma. Considering the 180.80 ± 5.30 ovules in one flower as well as the high natural seed set ($95.70 \pm 0.62\%$), this autonomous self-pollination contributed only slightly to selfing.

The self-pollination triggered by wind-driven corolla abscission occurs in late anthesis, varying from 0930 to 1130 h on different days depending on wind speed. Although the two pollinator species are usually active from about 0630 to 1100 h, they rarely appear after 0930 h on those days when the wind speed is over 2 m s^{-1} . In this species, the outcrossing resulted only from pollinator visitation. Therefore, when the self-pollination triggered by corolla abscission occurs after 0930 h, there are no opportunities for additional outcrossing. According to the definitions of Lloyd (1979), this self-pollination process belongs within the delayed self-pollination class. However, in the classification of Lloyd and Schoen (1992), delayed selfing is one of three autonomous modes of selfing (prior, competing and delayed), which occur without the participation of any external agent. In the present case, however, the wind plays a critical role in the process of corolla abscission and self-pollination. We therefore consider that the self-pollination triggered by corolla abscission in *I. sinensis* var. *sinensis* is a special mechanism of delayed self-pollination assisted by the wind.

In other taxa, delayed self-pollination can be achieved through the movements of floral parts at the end of anthesis, including downward curling of the stylar branches (*Campanula* spp., Faegri and van der Pijl, 1979; *Hibiscus laevis*, Klips and Snow, 1997), upward bending of the stamens (*Kalmia latifolia*, Rathcke and Real, 1993; *Aquilegia canadensis*, Eckert and Schaeffer, 1998; *Holcoglossum amesianum*, Liu et al., 2006) and changes in the corolla (*Mimulus guttatus*, Dole, 1990, 1992; *Pedicularis dunniana*, Sun et al., 2005). In all these cases, the delayed self-pollination was achieved autonomously without the involvement of any external agents even in *M. guttatus*, the only other case of delayed self-pollination facilitated by corolla abscission (Dole, 1990, 1992). However, the delayed self-pollination in *I. sinensis* var. *sinensis* involved the active participation of the wind. So the mechanism of delayed self-pollination found in this species is a new addition to the mode of self-pollination.

Lloyd (1979) and Lloyd and Schoen (1992) recognized that delayed selfing can provide reproductive assurance

when pollinators are unavailable. In the present study, only a few pollinators were observed visiting the flowers of *I. sinensis* var. *sinensis* despite extensive observations throughout the flowering season. During anthesis, only half of the flowers in the plots ($49.96 \pm 7.86\%$) received visits by pollinators. Similarly, $48.90 \pm 5.37\%$ of the flowers that were emasculated and exposed to pollinators developed into fruit, a figure significantly lower than that of untreated flowers under natural conditions ($85.93 \pm 1.63\%$). This indicates that selfing, including autonomous selfing before corolla abscission as well as delayed selfing facilitated by corolla abscission, contributed to the remaining approx. 37% of fruit production. Because the autonomous self-pollination before corolla abscission deposited only 66.9 ± 6.13 pollen grains (one-third of the ovule number), but the seed set attained $96.87 \pm 0.44\%$ in the flowers that were not visited by insects and allowed the corolla to abscise naturally, the delayed self-pollination triggered by corolla abscission contributed about two-thirds of the seed set when there was no pollinator visiting. This also indicates that the delayed self-pollination occurring in *I. sinensis* var. *sinensis* provides reproductive assurance when pollinators are absent.

Stigma closure in response to touch by an animal pollinator has been reported in some species of *Campsis* (Bertin, 1982; Yang, 2005), *Chilopsis* (Richardson, 2004a,b), and *Jacaranda* (Bittencourt and Semir, 2006) of Bignoniaceae. Yang (2005) reported that in *I. sinensis* var. *sinensis* populations located in Sichuan Province, the two open stigmatic lobes closed soon after pollinator touch and reopened after 15–30 min if no or a few pollen grains were deposited, but would permanently close after receiving many pollen grains. The present experiments confirmed this result and further revealed that the open stigmatic lobes did not close after receiving only pollen stimulation. Permanent stigma closure after being pollinated by insects prevented the delayed self-pollination triggered by corolla abscission, thereby reducing the interference of selfing with outcrossing within flowers (Webb and Lloyd, 1986). Reopening of the closed stigmatic lobes that receive no or only little pollen enables the ovules to be fertilized through the return visits of pollinators or the delayed self-pollination. Therefore, a sensitive stigma can be considered as a part of the mechanism of reproductive assurance, especially at the level of seed but not fruit production.

Ambophily

Incarvillea sinensis var. *sinensis* was pollinated by two bee species; and the wind also participated indirectly in the pollination process of this species through dragging the corolla to result in delayed self-pollination. The combination of both wind and insect pollination is defined as ambophily, which is considered to be selectively advantageous when there is temporal variation in pollinator abundance or pollinator scarcity (Vroege and Stelleman, 1990; Gómez and Zamora, 1996; Culley et al., 2002). In previous reports, wind pollinated entomophilous plants either through directly transporting pollen by air flow (*Hormathophylla spinosa*, Gómez and Zamora, 1996;

Schiedea lydgatei, Norman et al., 1997; *Calluna vulgaris*, Mahy et al., 1998), *Linanthus parviflorus*, Goodwillie, 1999; and *Salix* sp., Sacchi and Price, 1988; Vroege and Stelleman, 1990; Peeters and Totland, 1999), or through vibrating flowering stems to cause pollen dispersal (*Urginea maritime*, Dafni and Dukas, 1986). Moreover, ambophily generally occurs in those plants with small, simple flowers, some of which do not possess a salient perianth, such as *Salix* sp. In *I. sinensis* var. *sinensis*, the flowers have obvious features of entomogamy, possessing a large showy corolla, complex floral structure and a large volume of nectar. The wind provides pollination for this species only through dragging corolla to facilitate self-pollen deposition within flowers. This is also a new addition to the process of ambophily in angiosperms.

Anemophily requires that plants must possess certain floral morphologies or have an architecture that is conducive to wind pollination (Niklas, 1987; Culley et al., 2002). The flowers of *I. sinensis* var. *sinensis* possess a campanulate/funnel-form corolla with five large spreading lobes (mean 1.49 cm wide, 1.04 cm high), which provides a large area for the operation of the wind. In addition, the long axis of the flower is almost vertical ($<90^\circ$) with the rachis during anthesis, suggesting that gravity cannot play a key role in the process of corolla abscission. Moreover, anemophily also requires wind, as well as low humidity, low precipitation and open vegetation (Culley et al., 2002). According to the description of Yang (2005), *I. sinensis* var. *sinensis* generally grows in windy sites in Sichuan Province. In the current study area, the mean maximal wind speed during 0800–1200 h was above 2 m s^{-1} and rainfall seldom occurred from early July to the end of August. All of these conditions provide the probability for wind to drive corolla abscission and to participate in delayed self-pollination in *I. sinensis* var. *sinensis*. Because wind speed and rainfall are different in the various distribution areas of this species, the time of occurrence and frequency of this delayed self-pollination should be greatly variable.

In conclusion, in *I. sinensis* var. *sinensis*, the floral structure is highly specialized to achieve delayed self-pollination facilitated by corolla abscission. The process of corolla abscission driven by wind substantially enhances fruit and seed production by facilitating selfing, and provides reproductive assurance for this species. This mechanism may be an adaptation to variable pollinator abundance or pollinator scarcity in windy, rainless and open habitats. It would be interesting to know how this selfing pattern affects the mating system (selfing rate) in this species, and whether this pattern occurs commonly in close relatives of *I. sinensis* var. *sinensis* in similar habitats.

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