

Research Article

Pollen limitation and variation in floral longevity in gynodioecious *Potentilla tanacetifolia*

^{1,2}Hong YAO ¹Yi-Bo LUO*

¹(State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China)

²(Graduate University of the Chinese Academy of Sciences, Beijing 100049, China)

Abstract Pollination limitation is common in flowering plants and is thought to be a factor driving the evolution of floral traits. The plasticity of floral longevity to pollination may be an adaptation of plants to pollen limitation. However, this adaptation is less critical in short-lived flowers. To evaluate pollen limitation and the plasticity of floral longevity to pollination in *Potentilla tanacetifolia*, a gynodioecious herb with short-lived flowers, we analyzed its breeding system, tested sex-differential pollen limitation, and compared variations in floral display size in natural populations in Duolun County, Inner Mongolia, China. Hand pollination experiments and pollinator exclusion treatments revealed that *P. tanacetifolia* is self-compatible and non-autonomously apomictic and shows sex-differential pollen limitation. The plasticity of floral longevity to pollination was observed; the floral duration of female plants was prolonged by approximately 3–4 hours with pollination exclusion treatment. Moreover, the percentage of flowers displayed on female plants during pollination exclusion treatment was significantly higher than that during natural pollination. Under natural pollination conditions, the percentage of flowers displayed on female plants was significantly higher than on hermaphrodite plants. Furthermore, approximately 50% of the pollen grains spread out of the anthers of hermaphrodite flowers within 2 h of anthesis; the number of pollen grains adhering to the stigmas of hermaphrodite flowers was significantly higher than that adhering to female flowers when flowers shed their petals. These results indicate that variation in floral longevity may be an adaptive strategy to pollination conditions for gynodioecious *P. tanacetifolia*.

Key words floral longevity, gynodioecy, plasticity, pollen limitation, *Potentilla tanacetifolia*.

Insufficient cross-pollination is common in flowering plants because they are immobile and rely on various vectors to transfer pollen; however, plants can actively respond to pollination limitation, a condition under which inadequate pollen quantity or quality can reduce the reproductive success of plants. Some characteristics that influence fruit set, such as breeding systems (Arathi et al., 2002; Sato, 2002; Bartomeus & Vila, 2009; Marten-Rodriguez & Fenster, 2010), floral display size, and floral longevity (Ashman & Schoen, 1994; van Doorn, 1997; Rathcke, 2003; Harder & Johnson, 2005; Duan et al., 2007) may represent adaptations to pollination limitation.

The breeding system of a plant often determines the possibility or severity of pollen limitation (Lloyd & Schoen, 1992). Compared with obligate outcrossing species, self-compatible and autogamous species expe-

rience less intense pollen limitation (Larson & Barrett, 2000). Pollen availability is especially important in sexual dimorphic systems such as gynodioecy, in which female and hermaphroditic plants coexist in a single population, because it may influence floral evolution and relative fitness between the plants of both genders (Darwin, 1877; Ashman & Diefenderfer, 2001). In gynodioecy, female plants are expected to experience stronger pollination limitation than hermaphrodite plants due to the loss of male function and the possibility of self-compatibility (Maurice & Fleming, 1995). However, this difference is not always observed. For example, sex-differential pollen limitation does not occur at the level of individual flowers in the gynodioecious *Gypsophila repens* L. (Lopez-Villavicencio et al., 2003).

Floral longevity—the period during which a flower is fully open and functional—influences the amount and quality of pollen received and exported by the flower as well as the level of inbreeding (Primack, 1985; Ashman & Schoen, 1994; Harder & Johnson, 2005). An adaptation of plants, floral longevity varies greatly

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* Author for correspondence. E-mail: luoyb@ibcas.ac.cn; Tel.: 86-10-62836514; Fax: 86-10-62590843.

among species according to taxonomic background and their gender sequence type; the duration ranges from just a few hours to several days or weeks (Primack, 1985; van Doorn, 1997). Previous studies show that floral longevity is plastic in some species (Primack, 1985; Ashman & Schoen, 1994; Yasaka et al., 1998) and may be influenced by surrounding pollination conditions (Ashman & Schoen, 1994). Shortened floral lifespan is usually associated with a higher pollinator visitation rate (Stead, 1992; Ishii & Sakai, 2000; Stpiczynska, 2003; Abdala-Roberts et al., 2007). Due to the dependence of floral display size on individual floral lifespan, the floral longevity response to the abundance of pollinators would alter the number of flowers displayed simultaneously (van Doorn, 1997; Ishii & Sakai, 2001) and influence pollinator attraction (Eckhart, 1991; Ishii & Sakai, 2001).

The majority of studies regarding the plasticity of floral longevity focus on species with long-lived flowers (i.e. flowers lasting more than two days); fewer studies examine species with short-lived flowers (i.e. flowers lasting less than one day). The internal control of the timing of senescence is of considerable interest (Stead, 1992). Furthermore, it is proposed that flowers persist for long periods to compensate for a low frequency of pollinator visits (Primack, 1985; Ashman & Schoen, 1994). However, it is unknown how short-lived flowers respond to pollination limitation due to the absence of pollinators.

Potentilla tanacetifolia Willd. ex Schlecht. is a perennial gynodioecious herb with short-lived flowers that last for just a few hours during the daytime. It is distributed in Siberia, Mongolia, and some provinces of Northern China (Yu et al., 1985). The flowers require insect pollinators to transfer pollen. The flowering period of *P. tanacetifolia* starts in early July and ends in the middle of August in Duolun County, Inner Mongolia, China. Although pollinators are active during this period, pollinator unpredictability and pollen limitation can occur due to fluctuating weather. The objectives of the present work were to: (i) analyze the breeding system of *P. tanacetifolia* and evaluate whether pollen limitation occurs, particularly in female plants, in which male function is lost and pollination plays an important role in achieving seed fitness; (ii) explore whether pollination affects floral longevity in *P. tanacetifolia* and determine how its short-lived flowers respond to pollen limitation; and (iii) consider the adaptive significance of variations in floral longevity in gynodioecious *P. tanacetifolia*. To achieve these objectives, we carried out hand pollination experiments, pollinator exclusion treatments, and pollen dissemination and deposition examinations. Fruit set and variations in floral display size were measured

and compared among treatments. The number of pollen grains adhering to the stigmas was compared between genders.

1 Material and methods

1.1 Species and study area

Potentilla tanacetifolia is a perennial herb common in grasslands, mountain meadows, low-lying and sandy lands, and graveled areas (Yu et al., 1985). This species possesses a gynodioecious system, where female and hermaphrodite individuals are found in populations. Their inflorescences are corymbose-cymose and multiple flowers open each day. The diameter of the flowers is approximately 15–16 mm. The hermaphrodite flowers are homogamous and regular with five bright yellow petals, many pistils, and approximately 20 stamens. The anthers of female plants are either completely or partially reduced and are always non-functional. The fruit is an aggregate fruit consisting of one-seeded achenes. Individual flowers open between 08:00 and 09:00 and begin to wither between 13:00 and 15:00. The main pollinators are bees, and occasional visitors include dipteran-like syrphid flies.

Hand pollination experiments were carried out during the flowering period of 2006 and pollination exclusion treatments were carried out during 2007 in Duolun County (115°50'–116°55'E and 41°46'–42°36'N), a semiarid area of Inner Mongolia, China. Mean annual temperature is 2.1 °C with monthly mean temperatures of 18.9 °C in July and –17.5 °C in January. Mean annual precipitation is 385.5 mm with 80% concentrated from June to September (Niu et al., 2008). The study population was distributed in a typical agro-pastoral ecotone that is well conserved; livestock grazing had been excluded for five years. The major vegetation type is typical steppe.

1.2 Breeding system and pollen limitation experiments

To analyze the breeding system of *P. tanacetifolia* and test whether pollen limitation occurs at the level of individual flowers, we carried out the following hand pollination experiments on randomly selected individual flowers of 10–20 female and hermaphrodite plants in 2006: (i) cross-pollination of female flowers (female flowers pollinated with pollen from hermaphrodite plants and bagged; $n = 20$ treated individuals); (ii) cross-pollination of hermaphrodite flowers (bagged hermaphrodite flowers that were emasculated and hand pollinated with allopollen; $n = 30$); (iii) spontaneous self-pollination (bagged hermaphrodite flowers

without any treatment; $n = 20$); (iv) geitonogamy (bagged hermaphrodite flowers that were emasculated and hand pollinated with pollen from other flowers on the same plant; $n = 12$); (v) autonomous apomixis in hermaphrodite flowers (hermaphrodite flowers emasculated and bagged; $n = 20$); (vi) autonomous apomixis in female flowers (bagged female flowers without any treatment; $n = 20$); and (vii) control (open-pollinated flowers of both genders; $n = 30$). All flowers were labeled after treatment.

In experiments (i)–(vi), the emasculation and bagging processes were carried out at the start of anthesis in the morning to prevent natural pollination. Pollination treatments were carried out in several individual flowers on different days. All cross-pollinated flowers were hand pollinated with a fresh pollen mixture collected from at least 10 individuals. The parchment bag was maintained until the fruit matured; the aggregate fruit was subsequently collected, and fruit production was recorded. Fruit set was calculated as follows: number of mature achenes/number of carpels per flower \times 100.

1.3 Effect of pollination on floral longevity

To determine the effect of pollination on the floral longevity of *P. tanacetifolia* and assess the response of its short-lived flowers to pollen limitation, we carried out the experiments on the following types of flowering individuals in the population in 2007: (i) open-pollinated females; (ii) open-pollinated hermaphrodites; and (iii) pollinator-excluded females. On the sunny morning of 25 July 2007, 11 females were randomly selected and covered with a nylon net before anthesis; the distance between two individuals was at least 40 m. Near each covered female, uncovered control plants (one female and one hermaphrodite plant) were tagged. Records of individual floral display size started when the maximum display size was reached around 12:00. The floral display size was calculated separately for each individual every 1–2 h. The effect of pollination on floral longevity was evaluated by assessing the variation in floral display size, which was calculated as follows: number of flowers displayed/largest floral display size in a day \times 100.

1.4 Pollen dissemination and deposition on stigma

On the sunny day of 6 August 2006, pollen dissemination was determined by marking a random sample of flowers ($n = 40$) of several hermaphrodite plants ($n = 20$) at the start of anthesis, then harvesting a subset ($n = 5$) of these flowers at 1-h intervals over the flowers' lifetimes. Each time interval was repeated five times. For harvested flowers, the number of pollen grains remaining in anthers was determined. Random samples of flower buds were also collected to determine the number of pollen grains initially present in anthers. Raw data were transformed to reflect the accumulation of fitness on a proportional scale: the proportion of the total pollen grains per flower present at $t = 0$ that were disseminated after t hours.

To determine the number of pollen grains adhering to the stigmas, random samples of open-pollinated female flowers ($n = 19$), open-pollinated hermaphrodite flowers ($n = 19$), cross-pollinated hermaphrodite flowers (emasculation and open-pollination) ($n = 8$), and self-pollinated hermaphrodite flowers (pollinator exclusion treatment) ($n = 10$) from several plants were harvested when all of the flower petals were shed in the afternoon of 8 July 2007. In each flower, the number of pollen grains adhered to 5–6 stigmas was counted.

1.5 Statistical analysis

Differences in the mean values of fruit set, percentage of flowers displayed, and the number of pollen grains on stigmas were tested using one-way ANOVA with a post-hoc Tukey's test for both genders of the species. The α level was set at 0.05. All tests were carried out using SPSS version 11.0 (SPSS, Chicago, IL, USA).

2 Results

2.1 Breeding system and pollen limitation

The results of the hand pollination experiments showed that all of the unpollinated female and hermaphrodite flowers failed to set fruit. Furthermore, both self-pollination and geitonogamy resulted in fruit set in hermaphrodite flowers (Table 1). Supplemental cross-pollination significantly increased the fruit set of flowers of both genders (Table 1); however,

Table 1 Fruit set and its ANOVA of females and hermaphrodites in *Potentilla tanacetifolia* for different hand pollination treatments

	Open (%)	Apomixis (%)	Selfed (%)	Geitonogamy (%)	Crossed (%)
Females	63.1 ^a \pm 16.9 (30)	0 (20)	–	–	77.1 ^b \pm 9.5 (20)
Hermaphrodites	76.4 ^a \pm 12.0 (30)	0 (20)	67.6 ^b \pm 15.3 (20)	58.3 ^b \pm 3.7 (12)	84.6 ^c \pm 11.1 (30)

Data collected from hand pollination treatments undertaken in 2006, and are the mean \pm SD. Numbers in parentheses indicate the number of flowers treated. Treatments with the same superscript letters (a, b, c) in each line are not significantly different from each other. Crossed, emasculation and open-pollination; open, open-pollination; selfed, pollinator exclusion treatment; –, not applicable.

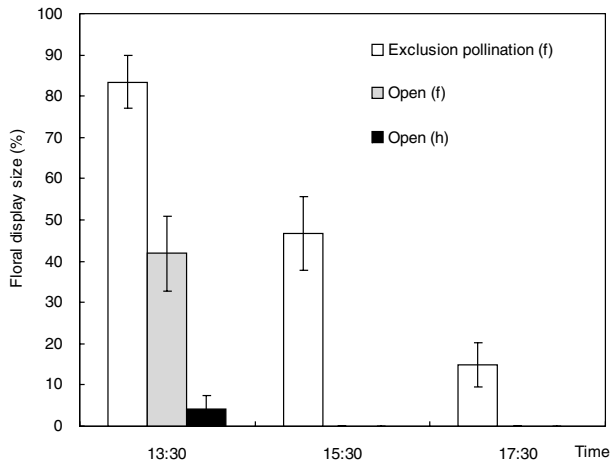


Fig. 1. Variations in floral display size (mean \pm SEM) for different pollination experiments of *Potentilla tanacetifolia*, observed 25 July 2007. f, females; floral display size (%), the number of flowers displayed/the largest floral display size in a day \times 100; h, hermaphrodites.

self-pollination and geitonogamy significantly decreased the fruit set in hermaphrodite flowers. In addition, no difference in fruit set between self-pollination and geitonogamy was observed (Table 1).

2.2 Effect of pollination on floral longevity

The results of the pollination control experiments revealed that the duration of floral display in female plants was prolonged for approximately 3–4 h when pollination was excluded (Fig. 1). The percentage of the flowers displayed differed significantly among the treatments at 13:30 on the day of observation ($F = 35.711$, $P < 0.001$) (Table 2, Fig. 1). At this time, more than 80% of the flowers were in bloom in the pollinator-excluded females; only approximately 40% and 4% of the flowers in the open-pollinated females and hermaphrodites were in bloom, respectively (Table 2, Fig. 1).

2.3 Pollen dissemination and deposition on stigma

The results of pollen dissemination showed that more than 80% of the pollen grains disseminated from 08:30 to 12:30; approximately 50% of the pollen grains spread out of the anthers within 2 h of anthesis. By 13:30, only 6% of the pollen grains remained in the

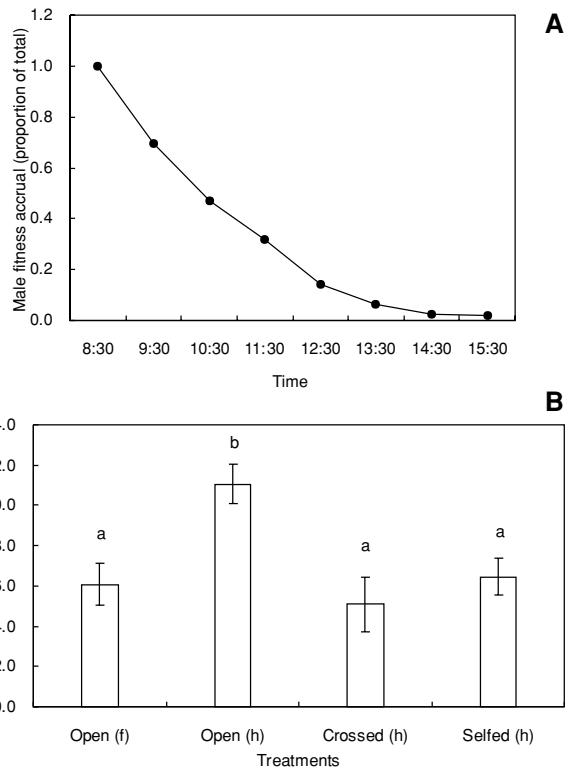


Fig. 2. Pollen dissemination and deposition in *Potentilla tanacetifolia*. **A**, Pollen dissemination of hermaphrodite flowers. Male fitness accrual, the number of pollen grains remaining in anthers per flower/the number of pollen grains initially present in anthers per flower. **B**, Pollen deposition on stigmas. Number of pollen grains on stigmas (mean \pm SEM). Treatments with the same letters above the means are not significantly different from each other. Crossed, emasculation and open-pollination; f, females; h, hermaphrodites; open, open-pollination; selfed, pollinator exclusion.

anthers; less than 2% of the pollen grains were left in anthers by 15:30 (Fig. 2:A).

When flowers shed their petals, the number of pollen grains deposited on the stigmas of open-pollinated hermaphrodite flowers 11.3 (\pm SD 4.4) was significantly higher than those on open-pollinated female flowers 6.2 (\pm 4.7), cross-pollinated hermaphrodite flowers (emasculation and open-pollination) 5.6 (\pm 3.9) and self-pollinated hermaphrodite flowers (pollinator exclusion treatment) 6.5 (\pm 2.9) ($F = 6.333$, $P < 0.001$) (Fig. 2:B).

3 Discussion

Plants hypothetically evolve toward reducing pollen limitation; furthermore, the floral traits selected may enhance pollinator attraction (e.g., larger floral display size) (Harder & Johnson, 2005) and/or lessen dependence on pollinators (e.g., obligate or delayed

Table 2 Analysis of variance of the floral display size in different pollination treatments of *Potentilla tanacetifolia*

	Mean \pm SD (%)	<i>n</i>	<i>F</i> -value	<i>P</i> -value
Open (h)	4.3 \pm 3.1	11	35.711	<0.001
Open (f)	41.8 \pm 30.3	11		
Exclusion pollination (f)	83.4 \pm 20.7	11		

Data collected at 13:30 on the day of observation. f, females; h, hermaphrodites; *n*, number of plants treated.

self-pollination) (Lloyd, 1992; Wheelwright et al., 2006; Kubota et al., 2008). Species with long-lived flowers generally produce relatively few flowers and require insect visits for seed production. In contrast, species with short-lived flowers produce many flowers that are capable of automatic self-pollination in the absence of pollinators (Ashman & Schoen, 1994). The flowers of *P. tanacetifolia* are short-lived, abundant, and gynodioecious.

3.1 Breeding system and pollen limitation

In hand-pollination experiments, unpollinated flowers failed to set seeds, indicating that autonomous apomixis is negative (Table 1). Some *Potentilla* species exhibit different ploidy levels; in addition, polyploid cytotypes are often facultative pseudogamous, in which embryo development may begin autonomously, but the endosperm only develops after fertilization (Goswami & Matfield, 1974). Only tetraploid *P. tanacetifolia* populations are found in Mongolia (Měsíček & Soják, 1992). Further investigation in this species is required to address the question of facultative pseudogamy.

In the present experiment, self-pollinated hermaphrodites set seeds, which indicates self-compatibility (Table 1). We observed that self-pollination took place after anthesis (Fig. 2:B), which may be a reproductive assurance in pollen limitation conditions. Specifically, an obligate outcrossing for females and a mixed system of self- and cross-fertilization for hermaphrodites was observed in this species. Moreover, in hermaphrodites, the increased fruit set that resulting from crossed pollination and the decreased fruit set resulting from self-pollination and geitonogamy primarily indicate the advantages of outcrossing (Table 1). Accordingly, in the gynodioecious system of *P. tanacetifolia*, females are apparently more likely to experience pollen limitation than hermaphrodites, due to their obligate outcrossing. Pollen limitation is often demonstrated empirically when supplemental pollination increases female fertility of open-pollinated flowers (Fox, 1992; Johnson & Bond, 1997; Knight et al., 2005). The results of hand-pollination experiments in the present study show that the fruit set in female flowers increases significantly after hand pollination (Table 1), suggesting that sex-differential pollen limitation occurs in this species.

3.2 Effect of pollination on floral longevity

Floral longevity is presumably optimized by natural selection in response to the pollination environment (Ashman & Schoen, 1994). In some species with long-lived flowers, the longevity of individual flowers is highly variable: the longevity of unpollinated flowers increases, whereas pollinated flowers have decreased

floral duration (Yasaka et al., 1998; Clark & Husband, 2007). In *P. tanacetifolia*, the duration of floral display in females was prolonged for approximately 3–4 h when pollination was excluded (Fig. 1). This suggests that pollination-sensitive floral longevity occurs in this species and floral duration may be extended in response to pollination limitation. Therefore, in *P. tanacetifolia*, although flowers last less than one day, the plasticity of floral longevity to pollination could also optimize the number of opportunities for receiving and exporting pollen.

3.3 Adaptive significance of floral longevity plasticity in *P. tanacetifolia*

Under natural pollination conditions, the percentage of flowers displayed in females was significantly higher than in hermaphrodites at 13:00 ($F = 35.711$, $P < 0.001$) (Fig. 1, Table 2). This indicates that in open-pollinated conditions, hermaphrodite flowers close sooner than female flowers in *P. tanacetifolia*. This difference may be due to coordination between floral longevity plasticity for pollination and sex-differential pollination. This explanation is supported by the results of the pollen deposition experiments, which showed that significantly more pollen grains adhered to the stigmas of hermaphrodite flowers than to those of female flowers ($F = 6.333$, $P < 0.001$) in open-pollinated conditions (Fig. 2:B).

The results of pollen dissemination indicate that more than 80% of the pollen grains disseminated between 08:30 and 12:30; approximately 50% of the pollen grains that spread out of anthers within 2 h of anthesis (Fig. 2:A). We observed that self-pollen grains spread out of the broken anthers and were spontaneously deposited on stigmas. When flower petals were shed, there were approximately $6.5 (\pm 2.9)$ pollen grains adhering to the stigma of self-pollinated hermaphrodite flowers (Fig. 2:B). In addition, we observed that pollinators visited flowers from sunrise to sunset. The results showed that there were approximately $5.6 (\pm 3.9)$ pollen grains adhering to the stigma of cross-pollinated hermaphrodite flowers and approximately $6.2 (\pm 4.7)$ pollen grains adhering to the stigma of open-pollinated female flowers (Fig. 2:B). Although there were no significant differences in the number of pollen grains on stigmas between cross-pollinated hermaphrodite flowers and open-pollinated female flowers, the sum of the number of pollen grains on stigmas of self- and cross-pollinated hermaphrodite flowers is similar to that of open-pollinated hermaphrodite flowers, which was significantly higher than on open-pollinated female flowers (Fig. 2:B). The behavior of pollinators might promote the pollination of both self pollen and allopollen for

hermaphrodite flowers, which may intensify the sexual difference as a result of pollination. In some gynodioecious species, hermaphrodites are more attractive to pollinators than females due to their larger floral display size and rewards of pollen or honey (Asikainen & Mutikainen, 2005). Therefore, additional self-pollination and/or differences in the attractiveness to pollinators possibly lead to the observed sex-differential pollination.

In gynodioecious systems, females must compensate for the loss of male function and improve the maintenance of their seed fitness (Darwin, 1877). It is obvious that although an outcrossing advantage may intensify female compensation in terms of seed quality, pollen limitation may reduce the female compensation in terms of seed quantity. It should be noted that in open-pollinated conditions, the variation in floral longevity, which mediates floral display size (Fig. 1), may influence the relative fitness of females. If the pollinators prefer visiting flowers with larger displays, the longer floral lifespan of females leading to larger floral display sizes would increase pollinator attraction and opportunities for outcrossing. Conversely, the shorter floral duration of hermaphrodites would be effective because it leads to a smaller display size, which decreases the cost of geitonogamous selfing. Therefore, in natural conditions, variation in floral longevity may be an adaptive strategy for reducing pollination limitation in females and increasing offspring fitness to some extent.

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