Research paper

Pollination adaptations of group-by-group stamen movement in a meadow plant with temporal floral closure

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Peer review under responsibility of Editorial Office of Plant Diversity.

ARTICLE INFO

Article history:
Received 27 July 2020
Received in revised form 29 March 2021
Accepted 5 April 2021
Available online 20 April 2021

Keywords:
Floral structure
Male-female interference
Pollen removal
Self-pollen deposition

ABSTRACT

Floral sexual organ (stamen and pistil) movements are selective adaptations that have different functions in male-female reproduction and the evolution of flowering plants. However, the significance of stamen movements in the spatial—temporal function and separation of male and female organs has not been experimentally determined in species exhibiting floral temporal closure. The current study investigated the role of slow stamen (group-by-group) movement in male-female sexual function, and the effect of stamen movement on pollen removal, male-male and male-female interference, and mating patterns of Geranium pratense, a plant with temporal floral closure. This species uses stamen group-by-group movement and therefore anther-stigma spatial—temporal separation. Spatial separation (two whorls of stamen and pistil length) was shown to be stronger than temporal separation. We found that stamen movements to the center of the flower increase pollen removal, and the most common pollinators visited more frequently and for longer durations during the male floral stage than during the female floral stage. Petal movements increased both self-pollen deposition rate and sexual interference in G. pratense. The fruit and seed set of naturally and outcrossed pollinated flowers were more prolific than those of self-pollinated flowers. Group-by-group stamen movement, dehiscence of stamens, pistil movement, and male-female spatial—temporal functional separation of G. pratense before floral temporal closure may prevent male-female and stamen-stamen interference and pollen discounting, and may increase pollen removal and cross-pollination.

1. Introduction

Morphological or spatial—temporal variation of stigma or stamen movement is important in the sexual interference and reproduction of flowering plants (Dole, 1990; Lloyd, 1992; Bynum and Smith, 2001; He et al., 2006; Abdusalam and Tan, 2014; Wang et al., 2017; Ye et al., 2019). The degree of spatial—temporal separation of floral sexual organs (herkogamy and dichogamy, respectively) can enhance male and female fitness in hermaphroditic flowers by preventing self-pollination and reducing interference between the functions of male and female organs (Barrett, 2002; Ren and Tang, 2012; Leite et al., 2016; Wang et al., 2017). The dramatic movements of floral structures that can separate male and female organs, such as flexistyly (e.g., the two phenotypes of Alpinia Roxb.), slow, or up and down, stamen movement in Ruta graveolens L., are thought to have evolved to promote successful reproduction in flowering plants (Li et al., 2001; Barrett, 2002; Yu and Huang, 2004; Von Hase et al., 2006; Wang et al., 2018).

Floral movements include both passive (non-directional and independent of ecological factors) and initiated (oriented response) movements (Ichmura and Suto, 1998; He et al., 2006; Ren and Tang, 2012; Wang et al., 2017), and they differ in function and adaptation in male-female reproduction and fitness (Ren and Tang, 2012; Abdusalam and Tan, 2014; Leite et al., 2016). Movement of flower
parts can reduce inter- or intra-sexual interference (Ren and Bu, 2014; Wang et al., 2017; Ye et al., 2019), influence mating patterns (increase the delay of self-pollination or cross-pollination) (Grant et al., 1979; Taylor et al., 2006; Du et al., 2012; Ren and Tang, 2012; Abdusalam and Tan, 2014; Wang et al., 2018), increase pollen transfer (Lewis, 1982; Lloyd, 1992; He et al., 2006; Song et al., 2013), and protect male and female organs under harsh environmental conditions (Schlindwein and Wittmann, 1997; Ren and Tang, 2012; Abdusalam and Tan, 2014). However, even in open flowers, the effectiveness of male organ functions may be increased by stamen movement (Ren and Tang, 2012; Wang et al., 2017; Ye et al., 2019).

Stamen movement is a key floral trait in reproductive fecundity, and it prevents interference between male-female sexual organs during the lifespan of hermaphroditic flowers (Schlindwein and Wittmann, 1997; Sandvik and Totland, 2003; Ren and Tang, 2012; Wang et al., 2017). Some plant stamen movement is induced by the filament or anther moving in response to pollinator activities or petal movement (Abdusalam and Tan, 2014; Wang et al., 2017), and is controlled by light and temperature (Henning and Weigend, 2012), growth of epidermal cells in the stamens (Du et al., 2012) and changes in the calcium content of cells that regulate water retention (Lechowski and Bialczyk, 1992; Cota-Sánchez et al., 2013).

Most flowering plant stamen movement (stimulated, simultaneous and slow, quick and explosive, and cascade) is induced by turgor pressure (energy from the flower) of cells in the filaments (Schlindwein and Wittmann, 1997; Taylor et al., 2006; Ren and Tang, 2012; Henning and Weigend, 2013). Stimulated and simultaneous stamen movement can result in self-pollen being deposited on the stigma, which increases self-pollination and female-male interference, e.g., in Berberidaceae (Lechowski and Bialczyk, 1992), and Calycanthaceae (Azuma et al., 2005). In contrast, quick, explosive and cascading (including one-by-one or group-by-group) stamen movements can increase pollen dispersal and out-crossing. For example, in Loasaceae, Moraceae, Parnassiaceae, Rutaceae and Tropaeoaceae, one-by-one stamen movements have an effect on pollen presentation in each flower (Taylor et al., 2006; Ren and Tang, 2012; Henning and Weigend, 2013). Stimulated and simultaneous stamen movement can result in self-pollen being deposited on the stigma, which increases self-pollination and female-male interference, e.g., in Berberidaceae (Lechowski and Bialczyk, 1992), and Calycanthaceae (Azuma et al., 2005). In contrast, quick, explosive and cascading (including one-by-one or group-by-group) stamen movements can increase pollen dispersal and out-crossing. For example, in Loasaceae, Moraceae, Parnassiaceae, Rutaceae and Tropaeoaceae, one-by-one stamen movements have an effect on pollen presentation in each flower (Taylor et al., 2006; Ren and Tang, 2012; Henning and Weigend, 2013).

2. Materials and methods

2.1. Study site and study species

Our experiments were conducted in a natural population of *Geranium pratense* growing in Bostanterak of Wucha County (39°25′20″−25°N, 75°09′28″−36°E, elevation: 2000–2500 m) in Xinjiang province, China, in July 2017 and 2019.

*Geranium pratense* is a summer-flowering herbaceous species that grows in grassland and alpine habitats at 1400–4000 m in Xinjiang Province, China. It produces a single umbellate inflorescence with 30–36 flowers that bloom in mid-June, while its fruits mature in early-August. Flowers of *G. pratense* have five sepal and the stigma at the center of the flower is surrounded by two whorls of five stamens each. These flowers respond to changes in light conditions, closing temporarily at dark, and reopening when light levels increase. In natural populations, stamen movement occurs in each flower at five floral stages (Fig. 1).

2.2. Movement of floral organs causes spatial and temporal separation of male and female floral functions

To estimate variation in anther and stigma spatial separation, the length of each stamen in both whorls and the pistil, and the vertical distance between each whorl of stamens and the stigma was measured in 30 flowers in each of the five floral stages: just-opened flower, outer whorl stamens dehisced, inner whorl stamens dehisced, style elongated, and stigma lobes expanded. All measurements were made (to the nearest 0.01 mm) on a sunny day using SE 2000 electronic calipers (Holy Instrument and Technology Co., Ltd; Guifin, China). To determine the duration of the five floral stages, 30 flower buds were marked, and the flowering stage was observed and recorded at 1-h intervals.

To determine temporal variation in pollen viability and stigma receptivity, 30 flower buds were covered with mesh netting to exclude pollinators. After the flowers opened, pollen viability at different floral stages was measured under a light microscope using the MTT method (Rodríguez-Riano and Dafni, 2000). To examine stigma receptivity, 100 flowers with non-dehisced anthers were selected, the anthers were removed, and flowers were then covered with mesh netting to exclude pollinators. At each of the five floral stages, 20 emasculated flowers were cross-pollinated by hand and then bagged with mesh bags. After a 3-week period during which the fruits matured, fruit and seed set were compared.

2.3. Pollinator type, visitation frequency and rewards

Pollinator visitation frequency, visitation duration, and behavior was determined at each floral stage. On a sunny day, 30 flower buds were randomly selected. After the flowers opened, insect visiting frequency and each insect during a foraging bout were observed for 10 flowers each in the outer male stage (outer whorl of stamens dehisced), inner male stage (inner whorl of stamens dehisced) and female (style open) stages of flowering. We photographed and caught 3–4 insects of each type for taxonomic identification. The following day, 20 flower buds were selected and covered with a mesh bag. After the flowers opened, the nectar volume was...
determined for the three flower stages according to the methods described by Dafni et al. (2005).

2.4. Effect of stamen movement on pollen removal rate

To assess the influence of group-by-group movement on pollen removal, we selected 30 plants with four mature flowers (flowers opened but anthers not dehisced) of the same size and at the same position of inflorescence. To establish a baseline level of pollen in these flowers, one flower from each inflorescence was selected, and the two whorls of stamens from each flower were placed together in separate 2-mL glass bottles (one bottle per flower) and taken to the laboratory. The other three flowers were assigned to one of three treatments: (1) filaments of two whorls of stamens tied together with style; (2) filaments of the two whorls of stamens tied together with the petals; and (3) filaments and stamens unmanipulated. After two days, the two whorls of stamens from these flowers were placed together in separate 2-mL glass bottles and taken to the laboratory. The number of pollen grains remaining on all anthers of each flower was counted under the Olympus BH-2 optical microscope (Nikon SMZ1000, Nikon, Japan) following the method described by Abdusalam et al. (2020).

2.5. Relationships between pollen removal rate and sexual interference

To determine the influence of stamen movement on the level of stamen–stamen interference and stamen–style position (interference), we selected 40 just-opened flowers (anthers not dehisced) in the natural population. Twenty flowers were allowed to be pollinated naturally and 20 flowers were covered with mesh bags to exclude insects. The position of the inner and outer whors stamens, and the distance between the style and the inner and outer whors stamens at different floral stages for each bagged and naturally pollinated flowers were measured using SE 2000 electronic calipers and photographs were taken. We recorded the angle and speed of stamen movement for bagged and naturally pollinated flowers. A protractor was used to measure the filament angle of the stamens in the inner and outer whors at five floral stages (just-opened flower; outer whorl stamens dehisced, when outer whorl stamens move to floral center; inner whorl stamens dehisced, when inner whorl stamens move to floral center; inner and outer whorl stamens dehisced; style elongated; and stigma lobes expanded) for each treated flower. When the filament angle was <10°, the stamens were considered moved away from the floral center (Ren and Bu, 2014).

To examine the effects of stamen and petal movement on stamen-stigma interference, four just-opened flowers (anthers not dehisced) on each of 40 randomly selected plants were assigned to one of four treatments: (1) filaments of outer whorl stamens were tied together with the style after removal of the inner whorl stamens (OF); (2) filaments of the inner whorl stamens were tied together with the style after removal of the outer whorl stamens (IF); and (3) filaments of both whorls stamens were tied together with the style (AF); or (4) no manipulation of floral organs (natural experiment, N). Twenty flowers for each treatment were selected and covered with mesh bags, and the other 20 flowers for each treatment were not bagged but anthers (and pollen) were stained a blue color following the procedure of Mamut et al. (2014). Two days after the initiation of each treatment, the stigma of each flower was placed in a separate 1-mL centrifuge tube, and the number of pollen grains deposited on the stigma was counted under a compound microscope in the laboratory following the method of Abdusalam et al. (2020).

2.6. Mating patterns

To determine the effect of stamen movement on mating pattern, we used five pollination treatments on 100 randomly selected just-opened flowers. Twenty flowers were assigned to each of five treatments: (1) natural pollination; (2) hand cross-pollination (i.e., all flowers were covered with paper bags after removal of all stamens, and the opened flowers were hand-crossed with pollen from plants >10 m away); (3) hand self-pollination (all flowers were covered with paper bags after the opened flowers were hand self-pollinated); (4) self-pollination (flower buds were kept covered

Fig. 1. Spatial interference between stamens of the inner and outer whors and between stamens of both whors and stigma in naturally pollinated (A–E) and bagged (no pollinator) flowers (F–J) of Geranium pratense at five floral stages: (A and F) just-opened flower; (B and G) outer whorl stamens dehisced; (C and H), inner whorl stamens dehisced; (D and I) style elongated; and (E and J) stigma lobes expanded.
with paper bags and flowers were allowed to self-pollinate); and
(5) controlled self-pollination (filaments of all stamens were tied
together with the style and flowers bagged with paper bags). Seed
production by flowers in each treatment was compared at fruit
maturation.

2.7. Statistical analyses

All data were tested for normality of variance prior to analysis to
fulfill requirements of a one-way ANOVA or independent samples t-
test. Percentage data (deposited self-pollen; removed pollen, fruit
and seed set) were arcsine transformed before statistical analysis to
ensure homogeneity of variance. Normal and homogeneous data
were subjected to further analysis, while abnormal and non-
homogeneous data were square root (percentage data, deposited
or removed pollen, filament angle and seed set) transformed before
analysis to ensure homogeneity of variance. Stamen and pistil
length, stamen-stigma distance, stigma receptivity, insect visiting
duration, nectar volume at different floral stage and deposited self-pollen and seed set for different treatment
flowers were compared using One-way ANOVA. If ANOVA indicated
significant differences, Tukey’s HSD test was performed for mul-
tiple comparisons to determine significant differences (p < 0.05)
among different floral stages. The length of the two whorls of sta-
mens and pistil, stamen-stigma distance, inner-outer whorl of
stamens distance, filament angle and deposited self-pollen in the
two flower treatments were compared using independent samples
t-test. Fruit set for different flower treatments was evaluated by the
generalized linear model (GLM) with Poisson distribution and log
linear-link function (Abdusalam et al., 2020). All statistical analyses
were performed using SPSS 19.0 software (SPSS Inc., Chicago, USA).
Non-transformed data appear in all figures.

3. Results

3.1. Spatial–temporal variation of male and female organ
separation

When centralized in the flower, the inner whorl stamens
(8.59 ± 0.53 mm) were longer than the outer whorl stamens
(7.96 ± 0.51 mm) (t = 7.514, p < 0.01, Fig. 2A). Stamen length in the
inner (F = 2.615, p > 0.05) and outer (F = 0.545, p > 0.05) whorls did
not differ significantly at different floral stages, but pistil length
differed significantly at different floral stages (F = 10.103, p < 0.01,
Fig. 2A). Inner whorl stamens were significantly longer than the
pistil at each flowering stage (F = 10.562, p < 0.01, Fig. 2A), as were
the outer whorl stamens (F = 4.653, p < 0.05, Fig. 2A). Male and
female organs were spatially isolated at different floral stages
(Fig. 2). The distance between both the inner and outer whorl
stamens and the stigma differed significantly at different floral
stages (t = 8.821, p < 0.01, Fig. 2B); however, inner and outer whorl
stamen length did not differ significantly at the female stage
(t = 2.041, p > 0.05, Fig. 2B).

Temporal variation in male and female viability is protandrous
(Fig. 2C). Pollen viability of outer (F = 13.19, p < 0.01, Fig. 2C) and
inner (F = 6.27, p < 0.01, Fig. 2C) stamens differed significantly from
stigma receptivity (F = 16.28, p < 0.01, Fig. 2C) at different floral
stages. Although pollen viability is higher than stigma receptivity at
male floral stage, stigma receptivity increases at floral closure and the
female stage. The duration of each floral stage differed signifi-
cantly (F = 125.19, p < 0.001, Fig. 2D). The duration of the female
stage (5.21 ± 0.75 h) was shorter than that of the male stages
(8.05 ± 0.26 h). In addition, the inner male stage (4.25 ± 0.31 h) was
shorter than the outer male stages (3.80 ± 0.21 h).

3.2. Pollinator type, visitation frequency and rewards

Eight types of insects visited Geranium pratense flowers (Fig. 3).
Most insect visitors probed the flower at the base of the pistil,
presumably seeking nectar, and often touched the floral center
(stigma or anthers). Pollinators included Apis mellifera, Bombus spp.,
Megachile sp., Colletes cuculianus, Amegilla nigricorni. Non-
pollinating visitors included Sideridis simplex, Syrphidae sp., and
Katamenes sesquicinctus. There were significant variations in polli-
nator assemblages and their visitation frequencies at different floral
stages. A. mellifera, Bombus spp., and Megachile sp. were the most
common pollinators, and visitation frequencies of these three
pollinators were higher at male stage than at the female stage
(Fig. 4A).

Nectar volume differed significantly at the three floral stages
(F = 3.911, p < 0.05). Flowers produce more nectar during the fe-
dmale stage (0.136 ± 0.009 vl/h) than during the outer (0.087 ± 0.014
vl/h) and inner (0.099 ± 0.015 vl/h) male stages. Pollinator visita-
tion frequency and foraging duration were significantly affected by
pollinator type (Fvisiting frequency = 8.06, p < 0.001; Foraging
time = 32.180, p < 0.001) and floral stage (Fvisiting frequency = 9.870,
p < 0.001; Foraging time = 12.012, p < 0.001, Fig. 4). Visitation fre-
cuency of all pollinators was higher at the outer male stage than at
the inner male stage (Fig. 4A). Foraging duration of pollinators was
significantly affected by pollinator type (F = 5.845, p < 0.01) and
floral stage (F = 3.732, p < 0.05). Foraging time of Bombus spp. and
A. nigricorni was longer at female stages than at both male stages.

3.3. Effects of stamen movement on pollen removal rate

The mean number of pollen grains in flowers prior to experi-
mental treatments was 3717 ± 174. Stamen group-by-group
movement had significant positive effects on the percentage of
removed pollen in the three treatment flowers (F = 19.478,
p < 0.001). The amount of pollen removed was higher for stamens
tied around the style (3489 ± 56) and flowers with unmanipulated
stamens (2990 ± 39) than in flowers with stamens tied together
with the petals (1229 ± 158).

3.4. Relationships between pollen removal rate and sexual
interference

The inner-outter stamen horizontal distance (F = 6.345,
p < 0.001) and filament angle (F = 11.039, p < 0.001) at different
floral stages differed significantly for natural (open-pollinated) and
no-pollinator (covered with mesh bags) flowers (Fig. 5). The hori-
Zontal distance of inner whorl stamen-stigma (F = 8.321, p < 0.001)
and outer whorl stamen-stigma (F = 7.359, p < 0.001) and filament
angle between outer and inner whorl of stamens (F = 8.532,
p < 0.001) differed significantly for naturally pollinated flowers and
those covered by mesh bags. The horizontal distances of outer and
inner whorl stamens were spatially isolated at different floral
stages in naturally pollinated flowers; however, during the inner
floral stage, there was interference between both the inner and
outer whorl stamens in bagged flowers (Fig. 5C and D).

The number of self-pollen grains deposited on the stigma
differed significantly in bagged and naturally pollinated flowers
(t = 12.257, p < 0.001, Fig. 6); more total pollen was deposited on
naturally pollinated flowers than on bagged flowers (Fig. 6A). Flowers in which the outer whorl stamens were tied together with the
style had less deposited self-pollen and less total pollen than
flowers in which outer whorl stamens were tied together with the
style (Fig. 6A and B). When the anthers were tied together with the
style, naturally pollinated flowers from the three treatments (OF, IF,
and AF) had more deposited self-pollen grains than did bagged
Fewer self-pollen grains were deposited on the stigma in unmanipulated flowers (i.e., stamens were not tied) than in treated flowers (i.e., anthers were tied with the style) that were bagged ($F = 10.156, p < 0.01$) or naturally pollinated ($F = 8.992, p < 0.01$, Fig. 6A). Stamen-style position and petal movement had significant effects on the percentage of deposited self-pollen, and the percentage was lower for unmanipulated flowers (i.e., stamens not tied) than for any of the three flower treatments (OF, IF, and AF, Fig. 6B).
3.5. Mating patterns

The fruit ($\chi^2 = 45.143, p < 0.001$) and seed ($\chi^2 = 15.861, p < 0.01$) sets for the five flower treatments differed significantly; they were larger for naturally pollinated and hand cross-pollinated flowers than for the three types of self-pollinated flowers (Fig. 7). However, the fruit ($\chi^2 = 3.045, p > 0.05$) and seed sets ($\chi^2 = 2.191, p > 0.05$) of the hand self-pollinated, automatic self-pollinated, and control self-pollinated (covered with a paper bag after the stamen was tied together with the style) flowers did not differ significantly. Automatic self-pollinated flowers did not set any fruit, which indicates the absence of spontaneous autogamy.

4. Discussion

4.1. Stamen movement reduces separation of anther-stigma functions

Our results indicate that stamen movement and pistil secondary growth in Geranium pratense flowers can greatly reduce the spatial–temporal separation between the two whorls of stamens and between the stamens and pistil. Stamen movement also decreases the separation between both whorls of stamens and the stigma attachment level. Spatial (herkogamy) and temporal (dichogamy) separation of the stamen and stigma can reduce...
interference between male and female functions in hermaphroditic flowers (Webb and Lloyd, 1986; Barrett, 2002; Ren and Tang, 2012; Armbruster et al., 2014). In *G. pratense* flowers, the lengths of stamens in both whorls remained the same and the position of each whorl changed at different floral stages, but the length of the pistil increased (secondary growth) during the floral lifespan (Fig. 2A). In addition, stigma receptivity and pollen viability were higher during the female than during the male flowering stages (Fig. 2C). The function of pollen viability and stigma in *G. pratense* was weakly separated temporally, and movement herkogamy (pistil) can be increased by sexual interference; that is, stamen group-by-group movement can decrease the interference between stamen-pistil temporal functions (pollen viability and stigma receptivity).

Our results suggest that in *Geranium pratense* there is increased interference between the inner and outer whorls of stamens when pollinator activity is low or anthers are not dehisced, and floral temporal closure occurs (Figs. 1E–J and 2C), as seen in other species. One-by-one stamen movement in *Parnassia palustris* and *R. graveolens* may be delayed if the pollen is not removed (Ren and Tang, 2012; Ren and Bu, 2014). If no pollinator exists, there could be a reduced amount of self-pollen deposited on the stigma after petal movement, and stamen movement decreases the amount of self-pollen deposited in the floral female stage (Figs. 1AB and 2C). Thus, stamen group-by-group movement appears to be an important floral characteristic that can increase pollen dispersal and anther-stigma spatial−temporal separation, as well as prevent viability of pollen deposits on the stigma of the same *G. pratense* flowers. As such, stamen group-by-group movement is likely an adaptation to the harsh grassland environment of this species that increases the pollen removal rate.

### 4.2. Effects of stamen movement on pollen removal rate

Our data indicate that stamen group-by-group movement to the center of *Geranium pratense* flowers in the first flowering day is crucial for pollen removal. Since potential pollen and nectar rewards are located in the central part of the flower, stamen movement to the floral center can increase pollen removal rate (pollen removal was greater for flowers with stamens tied to the pistil than for flowers with stamens tied to the petal). The floral center is the optimal position for stigma attachment to insect bodies and for the pollen reception rate, and stamen movement dramatically increases the accuracy of male function (Fig. 4), as seen in other species. In *P. palustris* and *P. epunctulata*, stamen one-by-one movement has also been shown to increase the amount of pollen removed (Ren and Bu 2014; Armbruster et al., 2014). In addition, most insect visitors probe the base of the pistil and often touch the floral center in *G. pratense*. In a controlled field experiment, the
percentage of removed pollen was higher in flowers with the filaments tied to the stigma than in those with no stamen movement. The group-by-group movement of the anthers can dramatically increase male function in *G. pratense*. For example, previous studies have shown that stimulated stamen movement can increase pollen removal to the insect body and the amount of pollen landing directly on the stigma rather than on the stamens (Grant et al., 1979). In addition, one-by-one movement of stamens not only decreases pollen discounting but also increases pollen removal in *R. graveolens*, *P. europulata* and *P. palustris* (Ren and Tang, 2012; Armbruster et al., 2014; Ren and Bu, 2014).

4.3. Relationships between pollen removal rate and sexual interference

Stamen group-by-group movement and pistil secondary growth (pistil movement) promote spatial—temporal separation of sexual organs in *Geranium pratense* flowers and prevent stamen-pistil interference. Floral temporal closure on the first day of flowering may reduce stamen—stamens and pistil-stamen interference. Some research has suggested that one-by-one stamen movement is a selective adaptation to prevent sexual interference in hermaphroditic flowers (Armbruster and Tang, 2012; Ren and Bu, 2014). Stamen movement to the floral central position of *G. pratense* after anther dehiscence reduces stamen-pistil interference, although low pollinator activity may be reduced between the inner and outer whorls of stamens. Often the stamens of the outer whorl will bend out of the floral center before those of the inner whorl. Successive stamen movements and patterns of style opening in the generalist pollinated *G. pratense* flowers may promote pollen removal by presenting pollen gradually to pollinators, and can prevent functional interferences between the stamen and pistil and between the inner and outer whorls of stamens. Therefore, group-by-group movement of stamens prevents stamen-pistil spatial—temporal interference; in contrast, one-by-one stamen movement, as reported previously in Loasaceae, Parnassiaceae, Rutaceae, and Tropeaeolaceae, prevents anther—anther interference (Ren, 2010; Henning and Weigend, 2012). The evolution of pistil movement and stamen group-by-group movement-dependent sexual spatial—temporal separation should be recognized as an important selective adaptation in *G. pratense* to the harsh grassland environment.

4.4. Stamen movement and mating patterns

Self-pollination and sexual spatial—temporal interference are achieved by flower non-morphological trait movements for some species (Abdusalam and Tan, 2014; Wang et al., 2018). For example, stamen movements result in automatic self-pollination in *Sanguinaria canadensis* L. (Lloyd, 1992) and *R. graveolens* (Ren and Tang, 2012). Petal closure (permanent closure) results in automatic self-pollination and ensures the reproductive success of *Chilopsis linearis* (Cav.) Sweet (Richardson, 2004; Von Hase et al., 2006). Our data indicate that *Geranium pratense* is a self-incompatible species (Fig. 6) and that group-by-group movement of stamens during the male stage before style opening can prevent self-pollination on the first day of flowering during floral temporal closure. Spatial and temporal separation of paternal-maternal functions can decrease deposition of self-pollen on the stigma and dramatically increase cross-pollination (Fig. 4). As the filament angle and the stamen-stigma distance decrease, the chance of self-pollen deposition on the stigma by insects decreases. Since self-pollinated and non-pollinated bagged flowers produced few mature fruits and seeds, we can conclude that seed set requires cross-pollination. If the anthers still contain pollen during the female stage, stamen movement to the floral center deposits the remaining pollen grains on the stigma. However, temporal separation of stigma opening can decrease the percentage of self-pollen deposited on the stigma; and if there is temporal and spatial separation of the stamen and stigma, sexual organ interference decreases and male and female reproduction increases.

5. Conclusions

Our study demonstrates that a staggered pollen removal level can reduce sexual interference and improve pollination accuracy. We found that temporal functions of male and female organs in *Geranium pratense* flowers were weakly isolated, but the function of both whorls of stamens was not temporally isolated. These findings indicate that group-by-group movement of the dehisced anthers, and spatial—temporal separation of the function of male and female sexual organs may increase pollen removal, prevent interference between whorls of stamens and between stamens and stigma, and decrease pollen discounting and self-pollen deposition, which may be selectively advantageous.

Author contributions

A.A. designed the study, analyzed the data, and drafted the manuscript. R.M. conducted field work, data collection and extraction. H.H. analyzed the data. G.A. conducted insect taxonomic identification work.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, “Pollination adaptations of group-by-group stamen movement in a meadow plant with temporal floral closure”.

Acknowledgments

This study was supported by the High-level Personnel Training Program of the Xinjiang Uygur Autonomous Region (QN2016BS0597).

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