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Dichogamy and style curvature avoid self-pollination in *Eremurus altaicus*

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This paper gives a systematic study of *Eremurus altaicus* in terms of flowering characteristics, pollinating features, style movement pattern, stigma receptivity and mating system. The result showed that it was protandrous and that the stigma had no receptivity until the end of pollen dispersal. Its style showed a regular movement pattern during the flowering phase. The style was upright, very close to anthers at first; then it quickly curved down 90° from the base just

before the dehiscing of anthers, but went back to the former upright state after pollen dispersal of all 6 anthers. From the blossoming to the end of pollen dispersal, the stigma was smooth and dry, and had no receptivity to pollens until the style went back to the upright state with papillae, and mucus appeared. The curving down movement of the style significantly widened the relative distance between the stigma and the dehiscing anthers. Therefore, protandry and style movement are double safeguard mechanisms for avoiding selfing and promoting outcrossing in *Eremurus altaicus*, which has important significance in its reproduction and evolution potential.

Keywords: Foxtail lily, mating system, protandry, pollination, style movement.

NUMEROUS studies suggest that generally plants avoid self-pollination and promote outcrossing. Dioecism, herkogamy, dichogamy and self-incompatibility are considered important mechanisms for angiosperm to avoid self-fertilization^{1–4}. However, self-compatibility and self-fruitfulness are viewed as adaptive strategies of reproduction assurance in habitats lacking effective pollinators^{5–10}. In the past few decades, several studies were done on the mating system and reproductive strategy of plants, and fruitful results were achieved^{11–16}.

Eremurus altaicus, the common name which is foxtail lily, is a herbaceous perennial of genus *Eremurus* (Liliaceae), mainly distributed in Central Asia, Siberia and China¹⁷. It was found only on the adrets of Tianshan Mountain and Altai Mountain (from 1000 to 2200 m amsl) in China. It has a raceme opened in sequence from bottom to top, and the styles of each floret showed a regular automatic movement of ‘erect → bend down → erect’ during the flowering stage leading to change in the spatial position and distance between the stigma and anther. However, development stages of the anther and stigma at different phases of style movement and ecological significance of the style curvature are still unclear presently.

Therefore, flowering characteristics, pollen dispersal features, mating system, anther dehiscence and stigma receptivity at different style movement stages of *Eremurus altaicus* were studied in this paper to reveal the adaptive significance of its style curvature.

From May 15 to June 25, in 2015 and 2016, fixed point observations (48°11.053’N, 87°01.482’E) of a natural *Eremurus altaicus* population (100 individuals) were performed at a grassland on the adret of Altai Mountain (at an altitude of 1066 m) in Burqin County, Xinjiang, where the perennial *Eremurus altaicus* (Liliaceae) (12 ovules in each floret) is a dominant species.

During 15 May–25 June in 2015 and 2016, we observed the whole flowering season of 100 foxtail lily individuals and single flower pollinating features, and recorded the time of initial, full and final flowering stage and the dehiscence of the six anthers.

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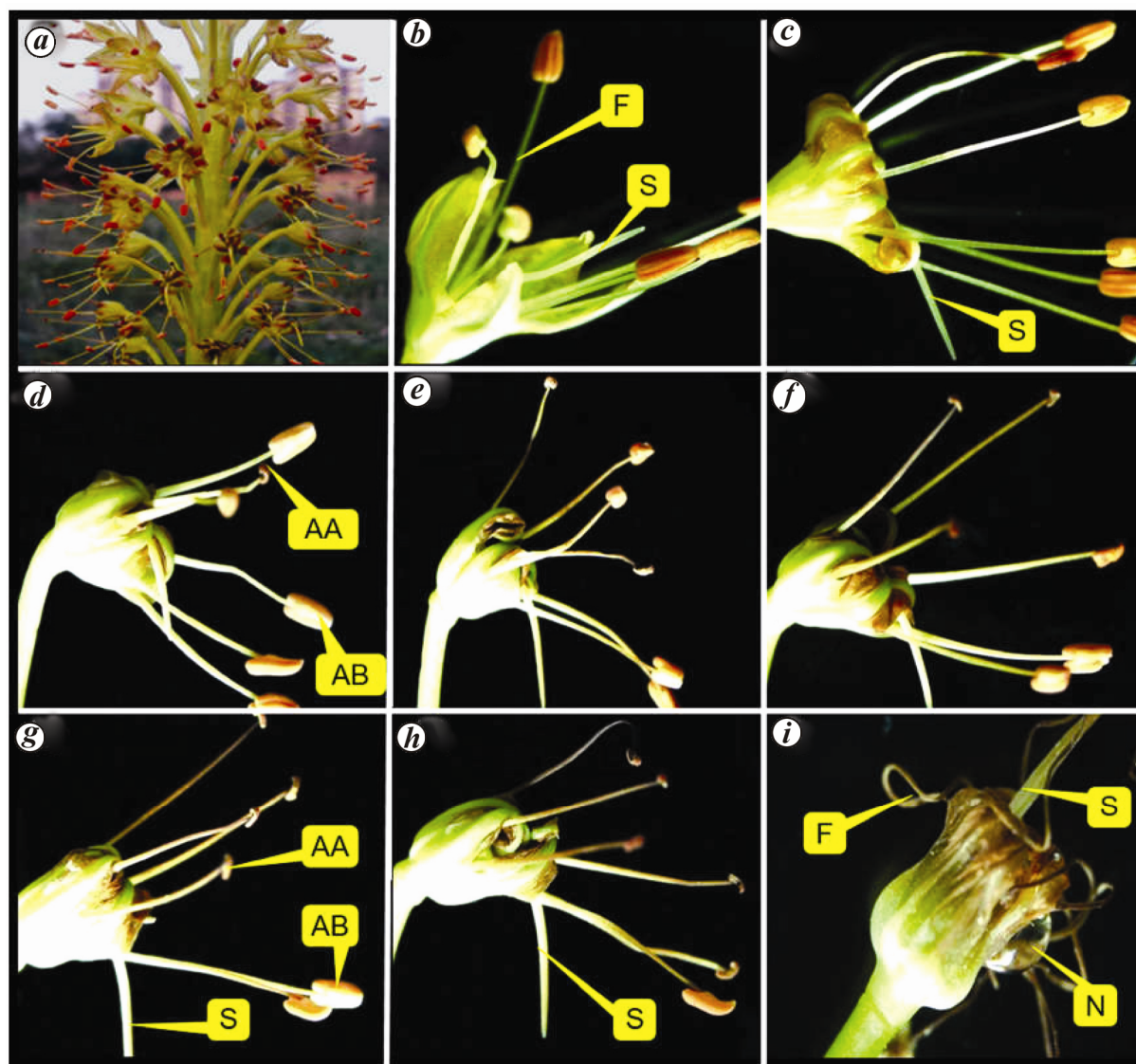


Figure 1. Inflorescence and style moving pattern of *E. altaiicus*. AA, Anther after pollen dispersal; AB, Anther before pollen dispersal; F, filament; N, nectar; S, style.

The pollen viability was determined by triphenyl tetrazolium chloride (TTC) method¹⁸. The pollens from dehiscent anthers of near-blossom flowers were scattered on clean glass slides at intervals of 20 min from the beginning of dehiscence of the first anther to the end of the last one. Then 1% TTC dye solution was dropped on each glass slide and quickly covered with coverslips. After 10 min, they were observed under an optical microscope to calculate the pollen viability. The red pollen grains in 3–5 visual fields were calculated under the microscope with 100× magnification. At least 200 pollen grains of each visual field were observed. Pollen viability = (red pollen number/200) × 100%.

After flowering, we observed and documented stigma position, style movement patterns and floral features corresponding to each movement phase of the 100 individuals.

Benzidine-hydrogen peroxide method¹⁹ was applied to detect stigma receptivity. Flowers that opened at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h were selected. Stigmas were immersed into benzidine-hydrogen peroxide reaction solution (1% benzidine: 3% hydrogen peroxide: water = 4:11:22) on the concave slides. If stigma was receptive, the reaction solution around the stigma turned blue with large number of bubbles appearing.

The stigmas selected (0, 2, 4, 6, 8, 10, 12 h after opening of the flowers) were fixed with FAA fixative solution for 48 h. The pollen grains on it were observed using scanning electron microscope after being dehydrated (ethanol solution gradient: 70%, 80%, 90% and 100%), dried and gold-stained.

At least 30 stigmas at each movement stage (upright stage I: from the beginning of flowering to the beginning of pollen dispersal; bend down stage: 0–7 h after

beginning of pollen dispersal; upright stage II: 8 h–4 d after beginning of pollen dispersal) were selected, fixed with FAA solution for 48 h, softened and transparentized with 8% KOH solution for 24 h, and tableted after 24 h dyeing using 0.1% water-soluble aniline blue (prepared with 0.033 mol l^{-1} , K_3PO_4 solution).

In the natural population, treatments were set as follows: control (without any treatment), bagged (the whole inflorescence was bagged before blossom), bagged after emasculation (all anthers in each bud were removed and then bagged), emasculated (all anthers in each bud were removed but not bagged), removal of tepals and stamens (both tepals and stamens in each bud were removed but not bagged). Ten individuals and no less than 30 buds of each were set for each treatment. After seed maturation, we calculated the mean fruit set rate of each capsule (seed setting rate = seed number/capsule number $\times 100\%$) and used one-way analysis of variance (SPSS19.0) to test differences of fruit set between treatments. To determine effective pollinators, we observed for 7 days continually (from 1 June to 7 June in 2016 and 2017) and recorded the visiting behaviour and visiting frequency of all insects, and detected whether *Eremurus altaicus*' pollens presented on the body surface of each visitor.

Eremurus altaicus' flowering season lasted about 20 days from 26 May (initial-flowering date) to 15 June (final-flowering date), and single flower longevity lasted about 3–4 days. The full-bloom stage lasted about 10 days (from 29 May to 9 June). Its flowers (6 yellow tepals) blossomed at about 8:00 am (anthers dehisced at 10:00 am), when the flower shape was broadly campanulate (Figure 1 a and b). About 24 h later, the top of the tepals began involuting and flower shape changed to become narrowly campanulate. The 6 anthers asynchronously matured and dehisced one after another, i.e. the next anther would not open until the former one completed its pollen dispersal (Figure 1 d–h). The pollen dispersion duration of single anther and single floret was about 60–70 min and 6–7 h respectively. The mean pollen viability at pollen dispersal stage was 88%.

In a floret, 6 stamens and 1 pistil were of similar length (Figure 1 a). The style was upright at the beginning of blooming (upright stage I: lasted about 2 h; Figure 1 b); then it rapidly curved down 90° from its base before involuting of tepal and dehiscing of anthers (bend down stage: lasted about 7 h; Figure 1 c). The style went back to its original erect state (upright stage II: lasted about 89 h) after the end of pollen dispersal of all 6 anthers and a large amount of nectar appeared (Figure 1 i).

The stigma had no receptivity during the bend down stage until the style went back to its former upright state (upright stage II). Its receptivity reached the highest level in the later 24–48 h and began to decline after 72 h. After 4 days, the stigma receptivity completely disappeared (Table 1).

During the whole pollen dispersal period (bend down stage), the stigma surface was dry, and no pollen was found on it (Figure 2 a). In the upright stage II, mucus appeared on the stigma surface with a number of pollens adhering to it (Figure 2 b).

No pollen was detected on the stigma until the upright stage II. After the style went back to the upright state, the pollen tube germinated on the stigma, passed through the style and entered the ovule finally.

Insects visited the control group frequently (7 times per hour on an average). *Xylocopidae* sp. and *Apis mellifera* L. were effective pollinators of foxtail lily since their bodies frequently contacted anthers and stigmas during the course of their visit resulting in a lot of pollen grains on their bodies. They often visited flowers from the top to the bottom, that is, they first visited the flowers at pollen dispersal, then flew down to the flowers which had completed pollen dispersal and had secreted lot of nectar. Nectar was the lure. Although flowers in pollen dispersal did not show significant nectar, the insects still searched for nectar at the base of the ovary. The large body size and high vibration intensity and frequency of their wings led to a lot of pollen falling down and attaching upon their bodies, thus assisting the outcrossing of *Eremurus altaicus*. The number of pollinators and visiting frequency decreased significantly after removal of stamens and tepals, and their fruit set rate was also significantly lower than that of the control (Table 2). This indicated that *xylocopidae* sp. and *Apis mellifera* L. were important pollinators, and yellow tepals, orange anthers and nectar were the main attraction^{20–22}. Seed setting in the treatment of being directly bagged, demonstrated that *Eremurus altaicus* was self-compatible, although its fruit set rate was significantly lower than that of the control (46.9%: 100%; $P = 0.001$). Apomixis was impossible for the plant since no seed was born in the treatment stamen removal and bagging.

Sex differentiation²³ and spatio-temporal distribution of flowers²⁴ have a profound impact on the mating system

Table 1. Variation in stigma receptivity of *Eremurus altaicus*

Time after pollen dispersal	Stigma receptivity
0 h	–
2 h	–
4 h	–
6 h	–
8 h	+/-
First day	++
Second day	++
Third day	+/-
Fourth day	–

++, Stigma is of highest receptivity; +/-, only part of stigma is of receptivity; –, stigma is not of receptivity.

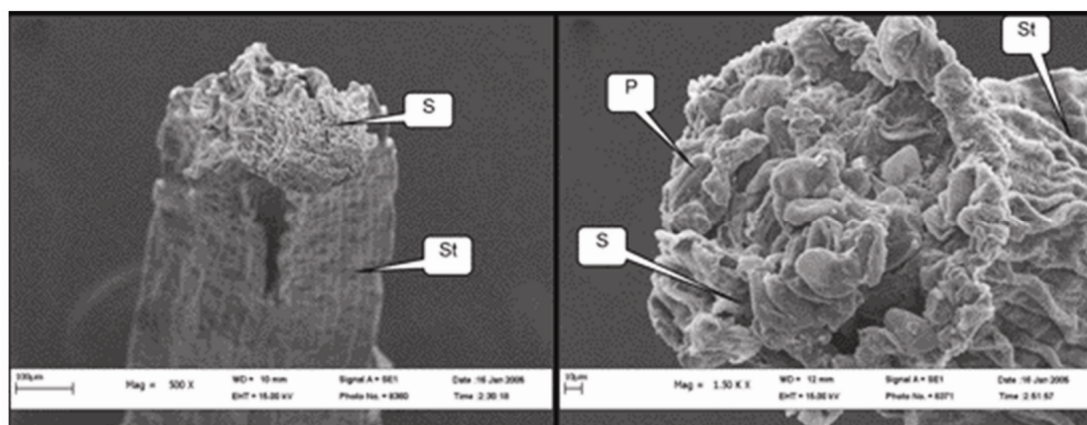


Figure 2. Stigma without and with pollens. A, Stigma without pollen; B, Stigma with pollens; P, Pollen; S, stigma; St, style.

Table 2. Comparison on average seed number per capsule among different treatments

Treatments	Average seed number per capsule
Control	4.37 ± 0.25 a
Bagged	2.05 ± 0.18 b
Emasculated	2.17 ± 0.31 b
Bagged after emasculation	0 d
Removing stamen and tepal	0.42 ± 0.11 c

Different English letters show significant difference ($P < 0.05$).

of plants, affecting the evolution prospect of the offspring population⁴. Unisexual flower is considered an effective mechanism for plants to avoid self-pollination²⁵. Structurally speaking, *Eremurus altaicus* flower is bisexual, but it is protandrous in the sense that the maturing time of the pistil and the stamen is completely isolated. Pollen dispersal was earlier than the stigma receptive period, and only after the completion of pollen dispersal did the stigma begin entering the receptive period. Since the male- and female-function periods of a flower do not overlap temporally, protandry is an effective mechanism for *Eremurus altaicus* to avoid self-pollination and inbreeding depression⁴.

Considering that the stigma's receptive period lasts about 3 days, self-pollination and self-fertilization might still occur when the stigma encounters pollens of other florets of the same raceme, at its receptive period. After bagging the whole inflorescence, it was found that all the buds could bear seeds, which suggested that *Eremurus altaicus* was self-compatible. This might be a reproductive assurance due to scarcity of pollinators⁵⁻⁸, which was consistent with the findings of *Eremurus anisopterus*²⁶.

Plants with style-movement are restricted to a few taxa, such as Zingiberaceae, Malvaceae, Marantaceae, Passifloraceae and Liliaceae, and the differences between their movement patterns are pronounced²⁷⁻³³. Because the style movement of *Eremurus altaicus* (erect → bent down →

erect) directly affects the position and distance between the stigma and anthers, it is bound to have a significant impact on the mating system of the foxtail lily. The style's curving away from anthers during pollen dispersal period can effectively widen the distance between the stigma and anthers, thus reducing the opportunity of the pollen grains falling on the stigma of the same floret. Dichogamy and style movement is the double safeguard mechanism for avoiding self-pollination in *Eremurus altaicus*.

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Antiproliferative and antibacterial activity of some *para*-substituted benzylideneacetophenones and establishing their structure activity relationship

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We report here *in-vitro* antiproliferative and antibacterial activity of *para*-substituted benzylideneacetophenones and established their structure activity relationship to optimize *para* position as a *biologically-oriented-synthetic* target for design of small molecule-based future anticancer/antibacterial agents. Among synthesized compounds, **1c** exhibits excellent antiproliferative activity against human osteosarcoma cell line (MG-63) compared to **1b** and **1a** suggesting dimethylamino (–N(CH₃)₂) functionality as a better *para*-substituted analogue for *in-future* anticancer agents. Similarly antibacterial screening of the aforesaid compounds against different strains of Gram-negative and Gram-positive bacteria reveals methoxy (–OCH₃) rather than dimethylamino (–N(CH₃)₂) as a better *para*-substituted functionality on ring B comparatively. From our results, we justify our theory ‘lipophilicity affects antibacterial activity’.

Keywords: Antiproliferative, antibacterial assay, benzylideneacetophenone, MTT assay.

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