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REGULAR PAPER

Growth discrepancy between filament and style facilitates autonomous self-fertilization in *Hedychium yunnanense* (Zingiberaceae)

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Background and aims – Reproductive success through autonomous self-fertilization is thought to be one of the main advantages in plants when effective pollinators are limited. The present study examined an autonomous self-fertilization mechanism in a perennial ginger, *Hedychium yunnanense*.
Methods – We measured the relative stigma-anther positional changes, throughout the flower's lifespan, in an effort to understand the floral mechanism that leads to autonomous seed production. Pollination treatments were carried out and pollen-ovule ratio was estimated to test its breeding system. Preliminary observations on floral biology and flower visitors were also conducted.
Key results – *Hedychium yunnanense* is a protogynous species and anther dehiscing occurred at night on the first day of flowering. The growth discrepancy between filament and style induces a contact between stigma and anthers favoring pollen deposition onto the stigmatic surface, thus facilitating self-pollination. Fruit set and seed set from pollinator-exclusion treatment showed similar results to open pollination treatment but lower than that of cross-pollination treatment. P/O ratio was 132.7 ± 13.4 indicating facultative autogamy. Effective pollinators were not observed in three consecutive years of observation.
Conclusions – The reproductive success of *H. yunnanense* via autogamy seems to be an adaptive strategy under the condition of effective pollinator limitation.

Key words – autonomous self-fertilization, pollinator limitation, growth discrepancy, *Hedychium yunnanense*, reproductive success.

INTRODUCTION

Reproductive success is a key process of seed plant life history. If a plant population relies solely on animal vectors to move pollen among individuals and if pollinators are absent or present in low numbers at certain times or years, individuals that can self-pollinate will possess a selective advantage (Lloyd 1979, Kalisz et al. 1999). Several observations are commonly cited as evidence for this; for example, by comparison with obligate out-crossers, species capable of self-fertilization tend to have wider geographical ranges, occur in marginal or temporary habitats, or have undergone major colonization episodes in their recent evolutionary history (Lloyd 1980, Eckert & Schaefer 1998). Moreover, autonomous self-fertilization can provide reproductive assurance when pollinators are absent or scarce (Lloyd 1979). Therefore, in inimical environments, reproductive assurance via autogamy is expected to be especially important, despite of the scarcity of empirical evidence (Stebbins 1957, Elle & Carney 2003).

Recent studies have found that many gingers are self-compatible (Classen 1987, Ippolito & Armstrong 1993, Kato et a1. 1993, Kato l996, Sakai et al. 1999, Gao et al. 2004, Gao et al. 2005). Some of these studies revealed that several species of Zingiberaceae displayed some unique pollination mechanisms (Li et al. 2001a, 2001b, Wang et al. 2004, Gao et al. 2005, Zhang & Li 2008). In the flowers of *Hedychium yunnanense* Gagnepain (Zingiberaceae), the position of the stigma, shortly after the flower fully opens, is higher than that of the anther. As time passes, the stigma is found to be at the same level, or indeed at a lower level than the tip of anther. Thus, the stigma easily touches the pollen when anther dehisces. In the present study, we focused on the understanding of this autonomous self-fertilization mechanism. In this respect, we measured the relative stigma-anther position changes over three days throughout the selected flowers' lifespan. Pollination treatments were designed and P/O ratio was estimated to test breeding system as well as flower visitors and preliminary floral phenology observations were also conducted.

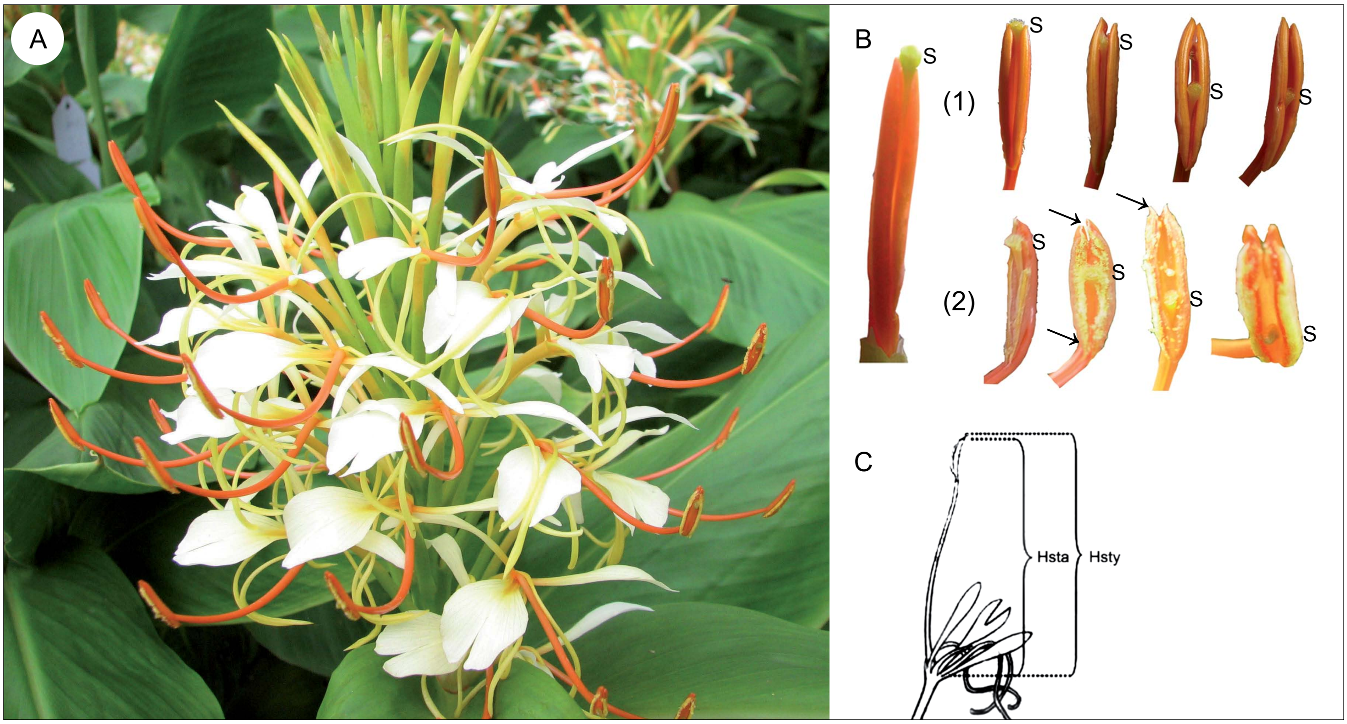

Figure 1 – A, an inflorescence of *Hedychium yunnanense* with various stigma-anther positions; B, the relative stigma-anther positions in different stage: (1) before pollen dispersal and (2) after pollen dispersal, with 'S' referring to stigma position. Arrows show that pollen grains are released all around the anthers; C, referring to the distance which Hsta and Hsty represent (the position of labellum connecting to filaments was used as the measurement reference point).

MATERIALS AND METHODS

Study species and site

Hedychium yunnanense is a perennial herb with a single inflorescence, often growing along evergreen broad-leaved forest margins. The average number of flowers per inflorescence was 45.90 ± 1.72 (mean ± s.e., n = 20). This species is distributed in Yunnan and Guangxi in China as well as in northern Vietnam (http://www.efloras.org). The flower's most significant character is the filament of the unique fertile stamen curving and forming a sheath in which the style grows. Anthers are linked by the red filament and the style is so slender that only through careful dissection of the filament can it be revealed. The corolla forms a slender tube with linear lobes. The linear oblong staminodes are shorter and wider than corolla lobes. Preliminary observations of floral biology were conducted from 2006 to 2008. Anther dehiscence time was recorded by observing 50 marked flowers in July 2007. In addition, corolla tube length, filament length, anther length, labellum length and width were measured, using vernier calipers, from a random selection of 20 flowers from 20 plants. Our experiments were carried out within the distribution range of *H. yunnanense* (> 200 plants) naturally growing at Kunming Botanical Garden (25°08'N 102°45'E, 1960 m a.s.l.), Yunnan Province, southwest China.

Measurements of relative stigma-anther positions

The relative growth rates of filament and style were measured, on 29 July 2006, in order to determine the changes of stigma-anther relative position. We randomly selected twenty flowers from ten individuals soon after the fully open phase of flowering. The position of labellum connecting to filaments was used as the measurement reference point (fig.1C). On each selected flower, we measured the height between the reference point and the tip of anther (Hsta) and the height between the reference point and the stigma (Hsty) at five times over a period of three days throughout the selected flowers' lifespan : t1 = 11:30 h and t2 = 16:30 h, on the first day; t3 = 09:00 h and t4 = 17:00 h, on the second day; t5 = 08:30 h, on the third day. All measurement process started at the same development stage. At the same time, fifty flowers were marked to detect the pollen viability and stigma receptivity using MTT (3-4,5-dime-Thylthiazol-2-yl-2,5-diphenyl-tetrazolium bromide, Sigma M-2128, Sigma, St. Louis, MO, USA) as described by Dafni (2001). Ten drops of pollen samples from each of ten flowers were evaluated, and for each drop, we counted at least 200 pollen grains in each of three random visual fields (Mao & Huang 2009).

Pollination treatments

Due to growth discrepancy between filament and style, *H. yunnanense* is easily self-pollinated. In July 2007, in order to test whether this species is self-compatible, flower buds from 20 individuals were randomly selected and subjected to the following four treatments: (1) bag directly, in which flower buds were bagged in order to avoid wind and animal pollinations. Bags were made of thick waterproof paper; (2) parthenogenesis test, in which flower buds were emasculated and bagged. (3) cross-pollination, in which flower buds

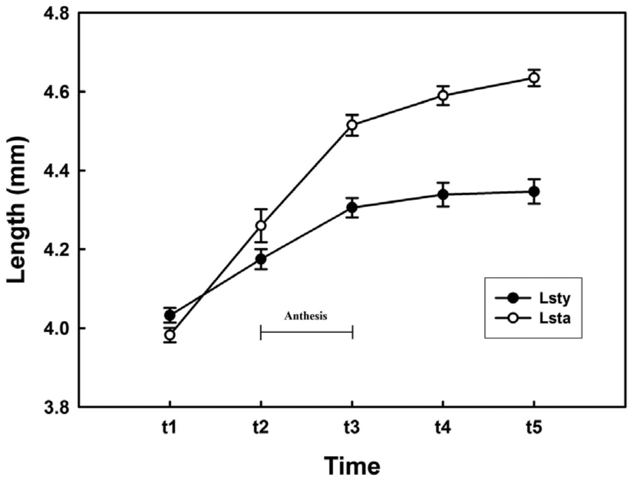

Figure 2 – The growth changes of Hsty and Hsta at five times over three days (n = 20), with dot line indicating the occurrence of anthesis.

were manually cross-pollinated with pollen from different individuals at least 3 m away; (4) open pollination, in which flowers were freely exposed to insect visitors. Fruit set was calculated as the ratio of mature fruits to the total number of manipulated flowers. Seed set was calculated as the ratio of expanded seeds to ovules per flower (Li & Huang 2009).

P/O ratio

The pollen-ovule ratio (P/O) was estimated following the method of Cruden (1977) and Dafni (1992). Twenty flower buds were chosen at random from ten individuals. The number of pollen grains and ovules in each flower were calculated and an average and standard error were estimated.

Flower visitors observation

We observed the floral visitors of *H. yunnanense* on four consecutive sunny days from 06:30h to 18:30h in July 2006, 2007 and August 2008. In July 2007, two night time observations, from 18:30h to 06:30h, were conducted to examine potential flower visitors. Insects which collected pollen and contacted stigmas were recorded as pollinators (Li & Huang 2009). All types of flower visitors including day and night were photo-recorded, identified and preserved in the insect collections of Kunming Institute of Botany, CAS.

Data analysis

All data was examined for normal distribution with a one-sample Kolmogorov-Smirnov test (Qu et al. 2007). One-way ANOVA analysis was used to compare differences in seed set among bagged, open pollination and cross-pollinated treatments. For paired-comparisons between treatments, post-hoc analysis was included. Data analysis was performed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA), and all presented data and figures are given as means ± standard error (s.e.), with *n* being the sample size.

RESULTS

Floral biology and the relative stigma-anther positions

On the basis of preliminary observation, the flowering period of *H. yunnanense* in the study site is from late July to mid-August. Flowers have a fragrant odor and flower longevity is 2-3 days. The average corolla tube is 4.74 ± 1.72 cm long and filament is 3.92 ± 0.07 cm long (n = 20). For anthers, the average length is 1.19 ± 0.03 cm (n = 20). The labellum is white and obovate, the average length and width is 2.65 ± 0.06 cm and 1.11 ± 0.04 cm, respectively (n = 20). The time of pollen release is always during the period from t2 to t3 (fig. 2). Pollen viability is 98.50 ± 0.44%, 96.00 ± 0.43% and 89.20 ± 0.99% at t3, t4 and t5 (n = 10), respectively. The stigma receptivity remained constant during the whole flowering time (from t1 to t5).

The position of the stigma is higher than the tip of anthers soon after the flowers fully open (t1): Hsty = 4.03 ± 0.02 cm; Hsta = 3.98 ± 0.02 cm (n = 20). From bud to the stage when flower is about to wither, Hsty and Hsta continue to grow. The last measurement (t5, third day) gives: Hsty = 4.35 ± 0.03 cm; Hsta = 4.64 ± 0.02 cm (n = 20). So, the position of the stigma is lower than that of the anther distal end. Therefore, due to different growth rates of filament and style, the relative position of stigma and anthers is changed. Growth changes of Hsty and Hsta during the observation period is represented on fig. 2. It is noted that pollen grains are released all around the anthers, indicated by arrows in fig. 1B. Hence, once the stigma is below the tip of anthers, it has the potential of self-pollination when anthesis occurs.

Pollination treatments

Fruit and seed set varied among the different pollination treatments. No fruit was found in emasculated and bagged buds (n = 31); therefore, the hypothesis of parthenogenesis is rejected. Fruit set from the pollinators-exclusion treatment was lower than that of the manually cross-pollinated flowers (59.1%, n = 115 vs. 86.8%, n = 76). Fruit set in open pollina-

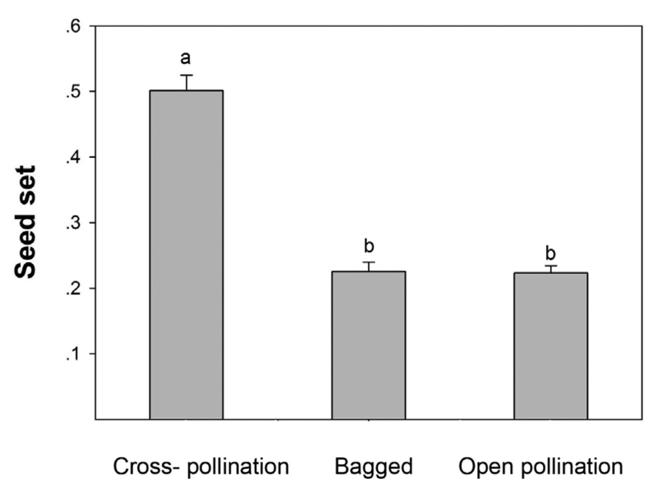

Figure 3 – Seed sets among cross pollination, bagged and open pollination treatments, with the same letter indicating no difference.

tion treatment was 62.3% (n = 69), which was similar to the bagged pollination treatment. Seed set of bagged flower buds is 0.23 ± 0.01 (n = 28) while seed set in open pollination is 0.22 ± 0.01 (n = 28), and no significant difference existed between the two treatments (F = 0.017; p > 0.05, fig. 3). However, seed set in cross-pollination treatment is 0.50 ± 0.02 (n = 20) and that is significantly higher than the other two treatments (F = 92.542; df = 2; p < 0.001, fig. 3).

Pollen-ovule ratio

There were on average 7894.00 ± 106.29 pollen grains and 60.05 ± 1.54 ovules per flower in *H. yunnanense* (n = 20). The P/O ratio was 132.69 ± 3.00, suggesting facultative autogamy according to the criteria established by Cruden (1977).

Flower visitor observations

In total, five types of insects were observed. In daytime, only hawkmoths (*Gurelca* sp.) and butterflies (*Papilio krishna*) visited the flowers. However, hawkmoths did not contact the stigma when foraging on flowers, and visit frequencies of butterflies were very low (only once during the observation period in 2007). At night, we found that three species visited flowers and carried pollen grains: scarab beetles (Scarabaeidae), *Episymploce sinensis* (Blattellidae) and *Tettigonia chinensis* (Tettigoniidae). However, they did not transport pollen successfully because they always stayed at the same flower. Therefore, all of the observed flower visitors were not considered as the effective pollinators.

DISCUSSION

Our observation reveals an autonomous mechanism for self-fertilization. Growth discrepancy of filament and style induces a contact between stigma and anthers. This is due to competing self-fertilization according to the classification of autogamy modes (Lloyd & Schoen 1992), because no effective cross-pollination was observed and thus it was not possible to distinguish self-pollination versus cross-pollination during anthesis. Furthermore, the elongation of stigma and anthers seems to continue throughout the flowering period, regardless of whether flowers were already pollinated or not (Ma et al., personal observation). To our knowledge, this is a new report of autonomous self-fertilization mechanism leading to reproductive success at least in the family of Zingiberaceae.

It is noted that pollinator movements are notably influenced by environment (Wilcock & Neiland 2002). The flowering activity of *H. yunnanense* lasts from late July to middle August, during the rainy season in Kunming city, which could contribute to the low frequency of pollinator movements. It is possible that effective pollinators could exist in natural conditions. Insects with a long proboscis are potential pollinators on the basis of floral characters; white labellum, fragrance odor and long filament as well as only a little nectar that is difficult to measure because of the long and fragile corolla tube with a very narrow inner space. In contrast, within the same genus, plants of *Hedychium coronarium* J.Koenig, which have a red labellum with similar fragrance odor, which were growing close by *H. yunnanense*, were frequently visited and pollinated by butterflies (*Papilio* sp.), and the position of the stigma was always above the anther throughout the flowering period (Ma et al., Chinese Academy of Sciences, China, personal observation). This illustrated the absence of an effective pollinator in shaping the behavior of autogamy. Selection for reproductive assurance is expected to be important for plant species when pollinators are rare (Stebbins 1950, Baker 1959, Goodwillie 1999). The lack of effective pollinators and no significant difference of seed production in bagged and open pollination treatments suggest that the autonomous self-fertilization is indeed selected and favoured for reproductive success in *H. yunnanens* growing in a botanical garden with frequent human disturbance.

This autonomous self-fertilization mechanism is an addition to the broad range of genetic and morphological self-pollination mechanisms in flowering plants, especially to the Zingiberaceae family. We compared representative behaviors of reproductive organs for pollination in Zingiberaceae: the stigma movement encouraged cross-pollination in species of *Amomum*, *Alpinia* while self-pollination occurred in *Roscoea schneideriana* (Loes.) Cowley (Li et al. 2001a, Zhang & Li 2008). Pollen movement induced self-pollination in *Caulokaempferia coenobialis* (Hance) K.Larsen (Wang et al. 2004). So, all of the strategies are involved in movements of either only male or only female reproductive organ. However, under the growth discrepancy process, both style and filament elongating movements induce self-pollination for reproductive success in *H. yunnanense*. A further study will be carried out with the intention of revealing more details of the pollination biology and understanding of the evolution of such a mechanism under different environmental selection for *H. yunnanense*.

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