

新西兰岩石百合中两种共存的繁育策略： 延迟自动自交和欺骗传粉吸引*

周 伟^{1,3}, 李德铎^{1,2}, 王 红^{1,2**}

(1 中国科学院昆明植物研究所生物多样性和生物地理学重点实验室, 云南 昆明 650201; 2 中国科学院西南
野生生物种质资源库, 云南 昆明 650201; 3 中国科学院研究生院, 北京 100049)

摘要: 延迟自动自交和欺骗传粉吸引在被子植物多数类群中有相关报道, 但是至今没有发现两种繁育策略在同一物种中共存现象。本研究通过对对照试验检测新西兰岩石百合雄蕊附属物和花冠闭合运动是否分别具有欺骗吸引和延迟自交功能。研究结果表明, 新西兰岩石百合黄色的雄蕊附属物拟态花粉 (或花药), 约 93% 的昆虫访花行为源自黄色花药附属物的欺骗吸引, 雄蕊附属物的报酬拟态功能有效提高昆虫拜访频率并促进异交。同时, 研究发现新西兰岩石百合花期结束时花瓣闭合促使雌雄隔离距离的缩小, 花瓣自然闭合的花朵平均结籽数 (20.62) 显著高于闭合前去雄处理花朵 (11.79)。我们的结果表明延迟自动自交与欺骗传粉吸引两种繁育策略在新西兰岩石百合中共存。

关键词: 延迟自动自交; 欺骗传粉吸引; 新西兰岩石百合

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Coexistence of Delayed Autonomous Self-pollination and Deceptive Pollination in *Arthropodium cirratum* (Asparagaceae)

ZHOU Wei^{1,3}, LI De-Zhu^{1,2}, WANG Hong^{1,2**}

(1 Key Laboratory of Biodiversity and Plant Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; 2 Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; 3 Graduate University of Chinese Academy of Sciences, Beijing 100049, China)

Abstract: Delayed autonomous pollination and deceptive pollination are assumed to be relatively common in flowering plants, but no species have been reported to use both of these cunning reproductive strategies. In this study, we examined whether delayed selfing and mimicry were used concurrently in *Arthropodium cirratum*. Flowers of *A. cirratum* were manipulated to assess whether their stamen appendages and corolla closing movements were functional in deceptive pollination and delayed selfing, respectively. Our results indicated that anther or pollen imitation of the yellow stamen appendages contributed to 93 per cent of successful mimetic attraction, and this mimicry was an important driver of cross-pollination of the species. In addition, we observed closure of the perianth relocated the stamens over the stigma at the end of anthesis, which significantly increased the average seed number per fruit of intact flowers over flowers emasculated before closure (20.62 versus 11.79). Our findings confirmed the coexistence of delayed autonomous self-pollination and deceptive pollination in *A. cirratum*. Our results also suggested that delayed selfing could add fitness benefits for this mimetic attraction species.

Key words: *Arthropodium cirratum*; Delayed autonomous self-pollination; Deceptive pollination

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** Author for correspondence; E-mail: wanghong@mail.kib.ac.cn

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作者简介: 周 伟 (1980-) 男, 博士, 主要从事植物繁育生物学与分子生物学方面研究。

Autonomous self-pollination, which occurs in flowers that are capable of outcrossing but in which selfing occurs without the participation of an external agent, is an important mating strategy to ensure sexual reproduction in harsh environments where pollinator services are unreliable. Lloyd and Schoen (1992) classified autonomous self-pollination into three general categories, termed 'prior', 'competing' and 'delayed', according to the timing of self-pollination relative to cross-pollination. Delayed self-pollination occurs after the opportunity to outcross has passed, and it may be achieved by either a partial overlap in the timing of male vs. female phases or by changes in the relative position of anthers and stigma within a flower during corolla movements (e.g. corolla abscission or closure). Delayed autonomous self-pollination is usually considered to be adaptive because it assures seed production when pollinators are scarce, yet allows cross-pollination to predominate when they are abundant (Cruden, 1977; Wyatt, 1983; Cruden and Lyon, 1989; Sun *et al.*, 2005; Fenster and Martén-Rodríguez, 2007).

Despite the selective advantage of delayed autonomous self-pollination, surprisingly few mechanisms are involved. For entomophilous plants, variation in pollinator frequency may be a significant force in the maintenance of mechanisms that induce autogamy late in floral life (Cruden and Lyon, 1989). Following this general idea, we raise the hypotheses that plant using mimetic attraction should be more likely to have evolved delayed selfing as a "bet-hedging" strategy since such plants may experience extremely unpredictable pollinator service due to their often rewardless, imperfectly mimetic flowers and the resulting learning-based cheating behavior of their pollinators (Mosquin, 1970; Menzel and Erber, 1978; Boyden, 1982; Ver-eecken and Schiestl, 2008). However, a link between mimetic attraction and delayed autonomous self-pollination in plants has not been described.

Preliminary observations of the "renga renga lily", *Arthropodium cirratum* (Asparagaceae), suggested that this perennial herb may utilize both mi-

metic attraction from its pollen-like stamen appendages and delayed autonomous self-pollination promoted by corolla closure. The flower of this species do not offer nectar and was scentless, but the yellow parts of the stamen appendages was always the target for visitors while the purple parts of the stamen appendages and the inconspicuous anthers themselves were mostly ignored. Late in flower development, the petals closed, the motion of which brought anthers and stigma together. Therefore, we designed this study to determine (1) whether the yellow stamen appendages attract pollinators to *A. cirratum*; and (2) whether the petal closure of the species triggers delayed autonomous self-pollination as a means of reproductive success. We tested these mechanistic hypotheses using detailed observations and manipulating flowers to measure pollinator visitation and seed production.

Materials and methods

Study system

Arthropodium cirratum is endemic to New Zealand and distributed across the Northern part of the island (Moore and Edgar, 1970). It was introduced to China in 1990 as an ornamental. This study was conducted at the Kunming Botanical Garden, Yunnan, China (KBG; 25°07'30" N, 102°44'17" E; altitude, 1 950 m a. s. l), where the species flowers from early May to late June, with the fruits maturing more than one month later. *A. cirratum* produces 1 to 4 inflorescences that bear more than 50 flowers each, and inflorescence are subtended by numerous leafy bracts. The Latinate term "cirratum" refers to the curled tendrils of the staminal appendage. These hair-like appendages can be divided into two types, the proximal yellow appendages and the distal purple appendages (Fig. 1C).

Mimic attraction experiment

To investigate the role of anther hairs in pollinator attraction, we recorded pollinator visits to four kinds of flowers: (1) intact flowers ($n=40$); (2) flowers with the yellow stamen appendages (YA) re-

moved ($n=40$); (3) flowers with the purple stamen appendages (PA) removed ($n=48$); and (4) flowers with anthers entirely removed ($n=40$). To reduce the possibility that the flower modifications would influence pollinator attraction at the inflorescence level, we combined three or four treatments within a single inflorescence, with each treatment applied to 2–5 flowers at randomized positions across the inflorescence. Pollinator visits were recorded at two time-intervals per day, coinciding with maximum pollinator activity, from 0800 hours to 1000 hours and from 1200 hours to 1400 hours during May 27 to May 29 and June 3 to June 4 in 2008. Visits were recorded each time a pollinator landed on any of the flowers, and a total of 148 flowers on 15 inflorescences were observed.

Delayed selfing experiment

To detect whether delayed autonomous self-pollination occurs during petal closure, we designed four treatments: (1) natural pollination as a control, the seed set of which was expected to be highest; (2) open pollinated and emasculated before the petals closed, the seed set of which would result from pollinator-carried pollen; (3) pollinator exclusion (using 1 mm×1 mm nylon netting), the seed set of which would be due to autonomous self-pollination following floral closure (given the absence of apomixis); and (4) apomixis, for which anthers were removed upon anthesis and flowers were covered with impermeable bags. Treatments were isolated on different inflorescences, and 20–30 flowers were manipulated in each treatment. One month later, we harvested 24 fruits in each treatment and counted the number of seeds per fruit.

Results

Continuous observation indicated that *A. cirratum* flowers opened between 0100 and 0200 hours, and closed between 1900 and 2000 hours. Thus the life-span of the flower was approximately 18 hours and never reopened after closure. The events during flower closure followed a rigid sequence with the in-

ner three petals closing before the outer three petals. During two observation days in which rain fell continuously, flowers opened at the same time for sunny days but petal closure was delayed by 2–3 hours.

Pollen-collecting bees and syrphid flies were the major visitors and pollinators of *A. cirratum* flowers, and no nocturnal visitor was observed when observation was made between 0100 and 0500. We recorded a total of 974 visits to flowers, of which bees and syrphid flies accounted for 98.5% (960 visits), the remaining 1.5% (14 visits) of visits were performed by butterflies and beetles. Mean visits per hour were 4.05 ± 1.12 (mean \pm s.d.), 0.28 ± 0.45 , 3.64 ± 1.01 and 3.86 ± 0.95 for treatments 1 to 4 respectively (Fig. 2A). Visits to intact (treatment 1), PA-removed (treatment 3) and anther-removed (treatment 4) flowers were not significantly different, but all three treatments had significantly more visits than for YA-removed (treatment 2) flowers (ANOVA, $F=150.77$, d.f. = 3, 144, $P < 0.001$, using Duncan's multiple comparison test; see Fig. 2A).

Flowers from all treatments except treatment 4 produced fruit (24 in each treatment). Mean seed number per fruit for treatment 1 (natural pollination) was 20.62 ± 5.28 ; treatment 2 (flowers emasculated before the petals closed) was 11.79 ± 4.61 ; and treatment 3 (automatic self-pollination) was 15.91 ± 5.94 (Fig. 2B). There were significant differences in seed number per fruit among the three treatments (treatment 1, treatment 2 and treatment 3; ANOVA, $F=16.61$, d.f. = 2, 69, $P < 0.001$). The results for treatment 4 (20 flowers) indicated that *A. cirratum* is incapable of apomixis.

Discussion

Because pollen grains of *A. cirratum* are sticky and apparently not moved by wind, successful reproduction relies on pollen transfer by its insect visitors. Our study showed that, although the flowers offer no reward at least no nectar, they attract pollen-collecting visitors by their conspicuous stamen appendages mimicking pollen-abundant anthers. How-

ever, the attractiveness of the two kinds of the appendages is asymmetrical, with high levels of attractiveness from yellow appendages (93%) but low attractiveness from the purple appendages (10%) (Fig. 2A). This result supports previous suggestions that most anther- and pollen-mimicking structures copy the deep yellow color of pollen and anthers, which represents a supernormal stimulus for most visitors, especially for bees (Lunau, 2000, 2006). In fact, we observed that bees and syrphid flies visit (but for few *Episyrphus balteatus* targeted at anthers see Fig. 1B) were predominantly to the yellow anther appendages, which brought the insects' bodies (abdomens) contact with anthers and stigmas

(Fig. 1A). This foraging behavior facilitated pollen transfer within or among flowers, with about 57.0 per cent of seed set (11.79 ± 4.61 versus 20.62 ± 5.28) coming from pollinator-facilitated self- and cross-pollination. Moreover, the comparison of seed number per fruit between pollinator-excluded and naturally pollinated flowers showed that at least 22.7 per cent of seed set ($1-77.3\%$, 15.91 ± 5.94 versus 20.62 ± 5.28) result from cross-pollination. Therefore, the pollen mimicry of the yellow stamen appendages appears to be an important driver of cross-pollination of the species.

The seed set of flowers emasculated before floral closure (11.79 ± 4.61) was significantly lower than

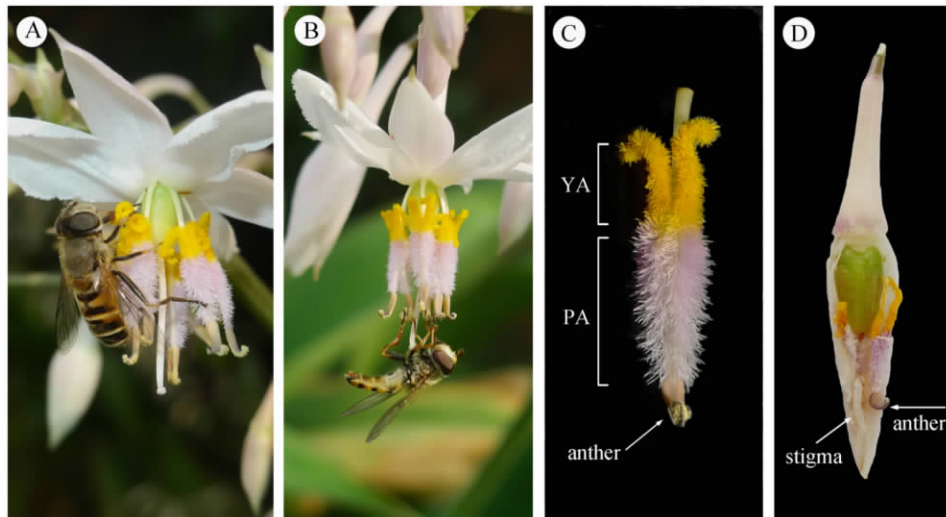


Fig. 1 Flower visitors and floral anatomy of *Arthropodium cirratum*. A, Mimetic attraction by yellow stamen appendages; B, Anther target visitation; C, Stamen structures. PA, purple stamen appendage; YA, yellow stamen appendage; D, Positions of anther and stigma in closed flower

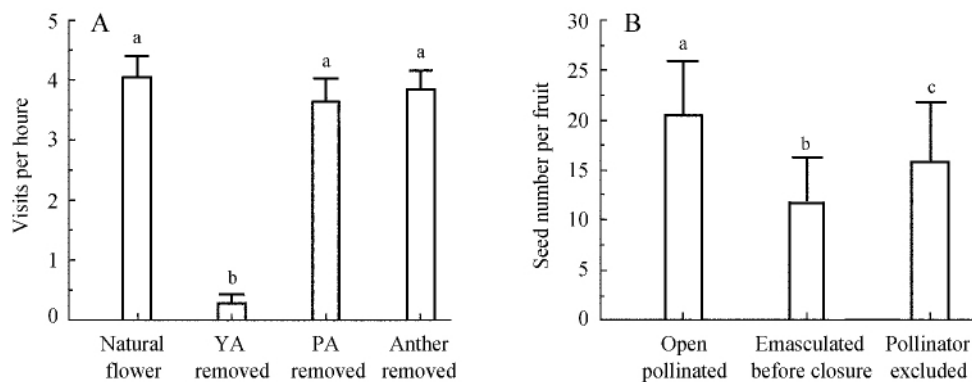


Fig. 2 Visit frequency and number of seed per fruit in *Arthropodium cirratum*. A, Numbers of bee visits per hour to flowers ($n=148$ flower) subjected to four experimental treatments; B, Seed number per fruit from the four pollination treatments ($n=72$ fruit). Different letter indicate significant differences between means by Duncan's multiple comparison test ($P<0.01$)

for flowers allowed to close naturally with intact anthers (20.62 ± 5.28 see Fig. 2B). This indicates that the increase in seed set associated with corolla closure results from delayed autonomous self-pollination. Selfed seeds were produced because closure of the perianth pushes the stamens over the stigma at the end of anthesis (Fig. 1D). This mechanism of delayed selfing is different from that of other species, such as *Kosteletzkya virginica*, in which delayed selfing results combination of stylar movements and corolla closure (Ruan *et al.*, 2005). Since at this study site, the species was cultivated in clusters and the pollinator visiting frequency was high (4.05 ± 1.12 per hour), the delayed selfing mechanism may be more crucial for naturally occurring *A. cirratum* growing in small, isolated populations (Fisher *et al.*, 1970; Parris *et al.*, 1971) where pollinator service may be limited and reproductive assurance may be required.

Delayed autonomous self-pollination and deceptive pollination are both assumed to be relatively common reproductive strategies in flowering plants, especially for species with unpredictable pollinator services and for orchid species, respectively (Schoen and Brown, 1991; Lloyd, 1992; Barrett, 2003). However, to our knowledge, this is the first report of delayed autonomous self-pollination also active in plants utilizing deceptive pollination. The delayed selfing could lend increased fitness benefits in this mimetic species. It would be interesting to know whether delayed selfing also contributes to reproductive success in other deceptive pollination species, since such plants may be more likely to suffer from reproductive failure and risk population loss or even species extinction.

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