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Research Article

How floral displays affect geitonogamy in an upward foraging bumblebee-pollinated protandrous plant

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Abstract Reducing geitonogamy (pollen transfer among flowers within the same plant) has been suggested as a major selective force for plants with multiple flowers. The occurrence of geitonogamy is generally different among flowers within inflorescences; however, no researchers have examined whether plants enlarge their display size without increasing the possibility of geitonogamy by presenting more flowers at positions where they are less likely to be geitonogamously pollinated. We observed that bumblebee pollinators foraged upward within the tower-shaped inflorescences of protandrous *Megacodon stylophorus* (C. B. Clarke) Harry Sm. Because *M. stylophorus* did not strictly bloom bottom-up, there were substantial frequencies of geitonogamous pollination resulting from upward and horizontal pollen transfer. Although there was a strong correlation between total numbers of flowers plants produced and numbers of flowers presented on single days, proportions of flowers possibly geitonogamously pollinated were weakly correlated with total numbers of flowers. This might have been because plants with more resources enlarged their display size by producing more flowers on lower floors where flowers had a low probability of being geitonogamously pollinated. This study shows that the tower-shaped inflorescences of *M. stylophorus* enlarge their size without more cost of geitonogamous mating, suggesting that geitonogamy acts as an important selective agent in the evolution of inflorescence architectures.

Key words: floral display, foraging behavior, geitonogamous pollination, *Megacodon stylophorus*.

1 Introduction

Plants displaying many flowers could attract more pollinator visits per plant and per flower than plants with few flowers, potentially importing and exporting more pollen grains (Klinkhamer & de Jong, 1993; Galloway et al., 2002). However, increasing attractiveness to pollinators induces longer visitation sequences, which often accelerates geitonogamous pollination (transfer of pollen among flowers on the same plant) (de Jong et al., 1993), and it has been suggested that the number of flowers to be geitonogamously pollinated exponentially increases with floral display size (de Jong et al., 1992; Karron & Mitchell, 2012). Self-fertilization resulting from geitonogamous pollen transfer could lower fitness due to inbreeding depression (Charlesworth & Charlesworth, 1987; Eckert, 2000). In addition, geitonogamy might reduce the amount of pollen that could otherwise have been exported (pollen discounting; Harder & Barrett, 1995; Lau et al., 2008), and the number of ovules available for outcrossing (ovule discounting; Herlihy & Eckert, 2002). Therefore, reducing geitonogamy has been suggested as a major selective force for plants with multiple flowers (de Jong et al., 1993; Snow et al., 1996).

Floral visitors usually follow an optimal and possibly consistent route within inflorescences, which results in the maximum net rate of food gain (Heinrich, 1975; Hainsworth et al., 1983; Jordan & Harder, 2006). Plants possibly minimize occurrences of geitonogamy according to their visitors' behavior by spatial and temporal arrangement of flowers (Iwata et al., 2012; Jordan et al., 2016). Bee pollinators usually tend to start foraging at lower flowers and move upward in vertical inflorescences (Darwin, 1877; Utelli & Roy, 2000; Fisogni et al., 2011; Zhao et al., 2016); the placement of older and thus female flowers below younger male flowers in many protandrous plants is thought to have evolved to reduce geitonogamy, because lower female-phase flowers would receive pollen from other plants whereas upper male-phase flowers would disperse pollen to other plants (Harder et al., 2000; Routley & Husband, 2003; Jersáková & Johnson, 2007; Liao & Harder, 2014). Moreover, the possibilities of being geitonogamously pollinated might be different among flowers within inflorescences whose architectures are less structured in many dichogamous species (Rademaker et al., 1999; Galloway et al., 2002), and geitonogamous pollination usually occurs more frequently on upper flowers than on lower flowers when bees forage upward on inflorescences (Harder et al., 2000; Jersáková & Johnson, 2007). Many plants produce tower-shaped inflorescences, and it is interesting to know whether these plants enlarge their display size without increasing the possibility of geitonogamy by presenting more flowers on lower floors.

Megacodon stylophorus (C. B. Clarke) Harry Sm. (Gentianaceae) is a perennial, self-compatible, and protandrous herb that is pollinated by bumblebees (Meng et al., 2012). The plants produce a tower-shaped inflorescence with 3–5 pairs of opposite branches laden with a dozen flowers (Fig. 1). We observed that inflorescences did not strictly bloom bottom-up and bumblebee pollinators usually began their visits at the bottom flower and moved up on inflorescences, suggesting that the plant species and bee pollinators provide a suitable system for testing the theory outlined above. In this study, we recorded inflorescence phenology and pollinator movements, and investigated the direction of pollen flow to evaluate possibilities of geitonogamy at different positions within inflorescences. We also tested whether *M. stylophorus* enlarged display size by producing more flowers at positions where flowers had low probabilities of being geitonogamously pollinated.

2 Material and Methods

2.1 Study site and species

Observations reported here were recorded from early July to late August in 2014 at Potatso National Park, Shangri-La County, Yunnan Province, China (27.86°N, 99.99°E, 3654 m a.s.l.). *Megacodon stylophorus* is a perennial herb that is native to the East Himalaya montane regions, usually distributed along streams, shrubs, or forest margins at elevations of 3000–4400 m a.s.l. It produces leaves in a basal rosette, and sends up a 60–170 cm branched stalk laden with approximately 12 flowers in flowering seasons. Flowering occurs from June and July, and fruiting from July to September. Pendulous and campanulate flowers (2–4 cm in diameter and 4–5 cm in length) are radially symmetrical with five pale yellow-green petals and green calyxes. Nectaries are located around the base of the gynophore. The fruit capsules are ovoid-ellipsoid, 2.2–2.5 cm in diameter and 3–4 cm long (Ho & Pringle, 1995; Ge et al., 2005). In 2014, the study population of *M. stylophorus* included ca. 200–300 flowering individuals distributed along a forest-meadow boundary. In 2015 and 2016, we observed limited individuals produce flowering stems at the site.

2.2 Floral display

We marked 60 flowering stems to record floral displays. The position and sexual phase of each flower on each plant were recorded daily during the flowering season. Floral development was clearly separated into six phases: flower opening, pollen shedding (male phase), termination of pollen shedding, movement of stamens, stigma lobe opening (female phase), and flower wilting (Meng et al., 2012). The duration of the male phase was calculated from the beginning of pollen shedding to the ending of pollen shedding, and the female phase from the opening of the stigma lobes to the stigma turning brown. Inflorescences had 3–5 pairs of opposite branches and an apical flower. A flower terminated each branch, and usually one or two flowers (middle flowers) were located at the middle of lower branches (Fig. 1). We defined the lowest pair of branches as the first floor and apical flowers as the last floor. A paired *t*-test was used to assess the significance of difference in durations of male phases and female phases. Pearson's correlation tests were used to determine whether inflorescences with more flowers lasted longer and presented more flowers on a single day. A linear regression analysis was carried out to determine whether the number of opposite branches or the number of middle flowers on lower floors contributed more to the total number of flowers on inflorescences.

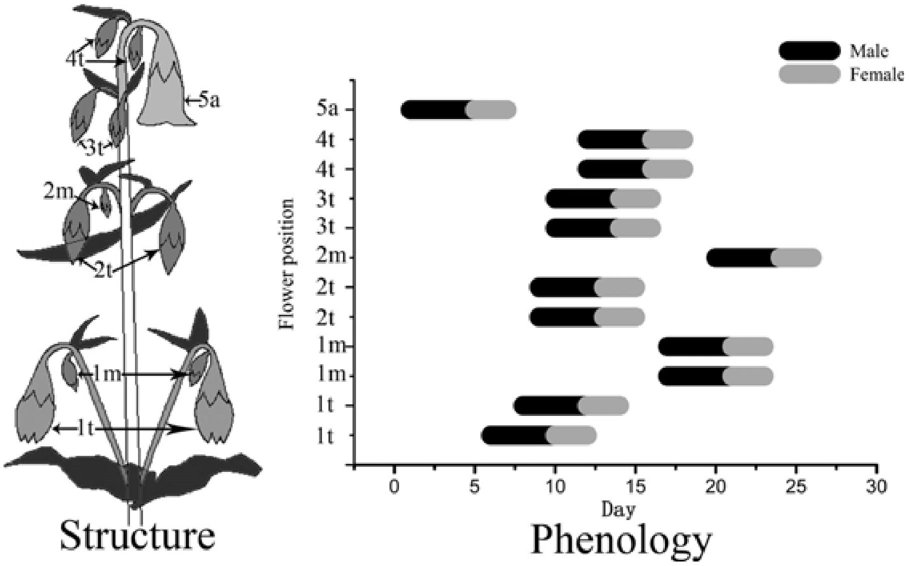

Fig. 1. Structure and phenology of a typical inflorescence of *Megacodon stylophorus* with five floors. Flower positions are described using a number and a letter indicating the floor and position (a, apical; m, middle; t, terminal) within it, respectively.

To examine variation of flowers' nectar production with time, we randomly selected 12 flower buds and bagged them in tulle nets in order to exclude visiting insects. We then monitored their development and measured the nectar volume and nectar concentration daily at 17:00 h. Nectar was drawn with 10-μL capillary tubes (Sigma-Aldrich, St. Louis, MO, USA), and volume was determined by measuring the length of the filled tube and converting the length measurements to microliters. The nectar sugar was measured using a hand-held, temperature-compensated refractometer (Bellingham & Stanley, Tunbridge Wells, UK). We undertook regression analyses with nectar secretion (volume and concentration) as the independent variables and the time as the dependent variable.

2.3 Observations of floral visitors

We observed floral visitors for 1–5 h after recording flowering phenology if the weather was not excessively wet or windy. Because flowering individuals were sparsely distributed, we focused on 1–2 inflorescences at a time for recording floral displays. All the floral visitors were recorded as well as their movement patterns within inflorescences and activities on flowers. Floral visitors were captured and preserved in the insect collections of Xishuangbanna Tropical Botanical Garden (Chinese Academy of Sciences, Mengla, China).

2.4 Direction of pollen flow within inflorescences

We used green or orange fluorescent powder (supplied by Shandong Jinan Langming, Jinan, China) to track pollen flow within inflorescences. The powder was mixed with a little water, and repeatedly added to dehiscing anthers of one male flower on selected inflorescences using a toothpick early in the morning. The next morning, we collected stigmas of flowers that had been in female phase on those inflorescences during the preceding 24 h. The stigmas were removed with forceps and placed individually in 1.5-mL Eppendorf tubes, and fluorescent particles on them were counted on the same day using a dissecting microscope (Leica M205A; Leica Microsystems, Heerbrugg, Germany). To reduce the possibility that observed fluorescent particles were caused by interinflorescence transfer, the treated inflorescences were spaced approximately 200 m apart, and we treated another inflorescence using the alternative powder at the same plot at least 5 days later. In pilot experiments no dye particles were found on fresh stigmas close to the experimentally treated inflorescences 5 days later ($n = 20$), as the female phase lasted approximately 3 days. Six terminal flowers and six middle flowers on lower floors (1–2) on separate inflorescences were dyed to mainly examine the possibility of upward pollen flow. In addition, six terminal flowers on upper floors (3–5) from different inflorescences were dyed, mainly to examine the possibility of downward pollen flow. On all the used inflorescences, if dyed flowers opened after their counterpart flowers (terminal flowers or middle flowers on the same floor), we collected stigmas of those counterpart flowers to test the possibility of horizontal pollen flow. In total, we obtained 17, 13, and 12 stigma samples to test possibilities of pollen flow upwards, downwards, and horizontally within inflorescences, respectively, and applied Fisher's exact test to evaluate the difference in pollen flow within inflorescences among the three directions. We also determined positions and numbers of flowers that were possibly geitonogamously pollinated; for these individuals, we monitored floral displays according to the result of intra-inflorescence dye transfer patterns. A G test was applied to evaluate the difference in possibilities of geitonogamous pollination between flowers located on the lower floors (1–2) and the upper floors (3–6). A linear regression analysis was applied to determine how the number of flowers possibly geitonogamously pollinated varied with the total number of flowers on inflorescences. In addition, Pearson's correlation coefficient was calculated to quantify the relationship between the total flower number on inflorescences and the percentage of flowers that could possibly be geitonogamously pollinated.

3 Results

3.1 Floral display

Four inflorescences were broken before flowering ended and thus were excluded from analysis. The other 56 monitored inflorescences included 689 flowers. Once flowers opened, pollen started shedding after several hours and stigmas remained closed. The male phase lasted 4–5 days. After that, anthers with the filaments moved towards the corolla and were finally next to it within 1 day, at which time the two stigma lobes opened. At the end of the female phase, the color of the stigmas changed from green to brown and corollas became putrid. We observed no overlap between male and female phases in any monitored flowers. The female phase lasted 2–4 days, significantly less than the male phase (paired *t*-test: $t = 74.93$, $d.f. = 688$, $P < 0.001$). The flowers produced 135.6 ± 34.1 μL of nectar on average (mean ± standard error). Regression analysis showed that there were reductions with time after flower opening in nectar volume ($R^2 = 0.52$, $P < 0.001$, $n = 106$) and sugar concentration ($R^2 = 0.55$, $P < 0.001$, $n = 106$) (Fig. 2). Flowers in male phase produced 2.13 times more nectar volume and 3.24 times more sugar per day than flowers in female phase.

Inflorescences produced 3.89 ± 0.09 pairs of opposite branches and opened 12.30 ± 0.55 flowers in total (6–22). Inflorescences lasted 24.98 ± 0.45 days (17–31), and the number of flowers on inflorescences was significantly correlated with their duration (Pearson's correlation: $r = 0.48$, $n = 56$, $P < 0.01$). Inflorescences presented 3.62 ± 0.06 flowers per day on average during their flowering periods, and 7.02 ± 0.29 flowers at peak bloom. The number presented on single days was correlated with the total number of flower inflorescences produced (Pearson's correlation: $r = 0.92$, $n = 56$, $P < 0.01$). In all the monitored inflorescences, the apical flowers always opened first. Most terminal flowers of the first floor opened when apical flowers were still in female phase. Terminal flowers of the first floor of only seven inflorescences opened after the apical flower wilted. Terminal flowers matured 1–4 days after terminal flowers on the adjacent lower floor. Middle flowers in the first floor usually opened when terminal flowers in the upper floors (3–5) were in female phase, and middle flowers in the second floor opened 1–3 days after middle flowers in the first floor.

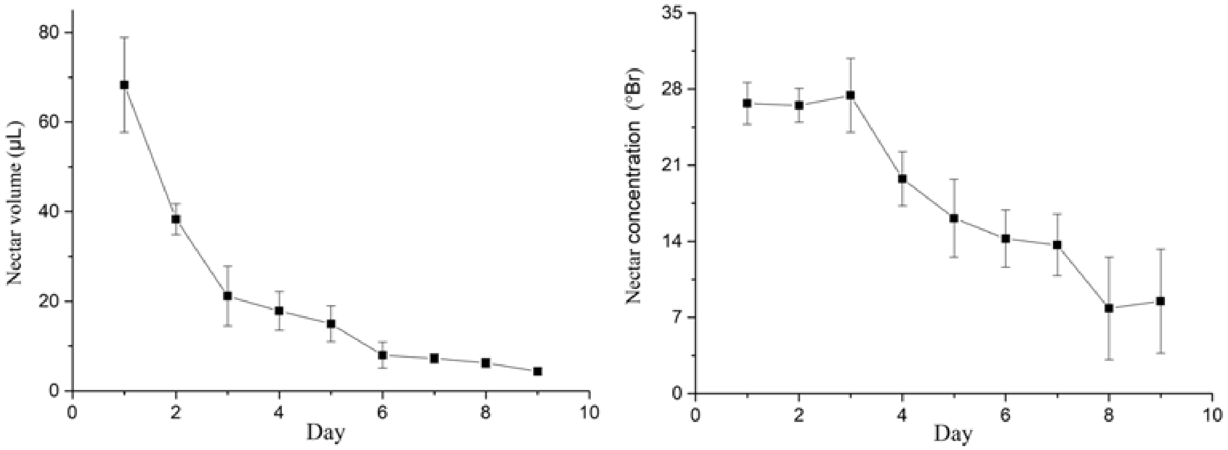

Fig. 2. Pattern of nectar secretion of *Megacodon stylophorus* flowers.

Counterpart flowers usually displayed synchronously, but sometimes there were 1 or 2-day differences between flowering times among them (Fig. 1). According to the linear regression analysis, numbers of middle flowers and opposite branches accounted for 93.5 and 5.90% of the variance in total numbers of flowers on inflorescences, respectively ($n = 56$, $R^2 = 0.994$, both $P < 0.01$).

3.2 Floral visitors

We observed floral visitors on 12, 13, 14, 20, 25, and 29 July, and 197 visits to inflorescences were recorded, with a total duration of 28 h. The main visitors were *Bombus richardsi* (156 visits) and *B. lucorum* (38 visits). *Bombus richardsi* visited flowers for nectar and in most cases (136 visits) they started visiting lower flowers and moved up within inflorescences. We also observed that *B. richardsi* only visited one or two flowers on the same floor (14 visits), or mainly moved upward but revisited one to two flowers on lower floors (6 visits). *Bombus lucorum* collected pollen grains from flowers and actively searched for flowers in male phase and never entered flowers in female phase. In addition, we observed two visits of a pollen-collecting solitary bee (Fig. 3).

3.3 Pollen flow within inflorescences

No fluorescent powder was found on any of the 13 stigmas used to assess downward pollen flow. However, we found fluorescent powder on 15 of 17 and 9 of 12 stigmas used to test upward and horizontal pollen flow, respectively, and the probability of these stigmas receiving the powder did not significantly differ (Fisher's exact test, $P = 0.62$). According to the results above, upward and horizontal pollen flow were quite possible. Within inflorescences used to monitor floral displays, 3.16 ± 0.42 (0–7) flowers in female phase were above at least one flower in male phase and geitonogamous pollen grains were possibly deposited on their stigmas through upward pollen flow. In addition, 1.43 ± 0.19 (0–4) flowers were in female phase while their counterparts were still in male phase, so geitonogamous selfing possibly occurred on them through horizontal pollen flow. Most apical flowers were possibly geitonogamously pollinated through upward pollen flow, and a large proportion of terminal flowers on upper floors were possibly geitonogamously pollinated both by upward pollen flow and horizontal pollen flow. However, only a minor portion of flowers on lower floors were possibly geitonogamously pollinated, mainly by horizontal pollen flow (Table 1). Generally, flowers on upper floors of inflorescences

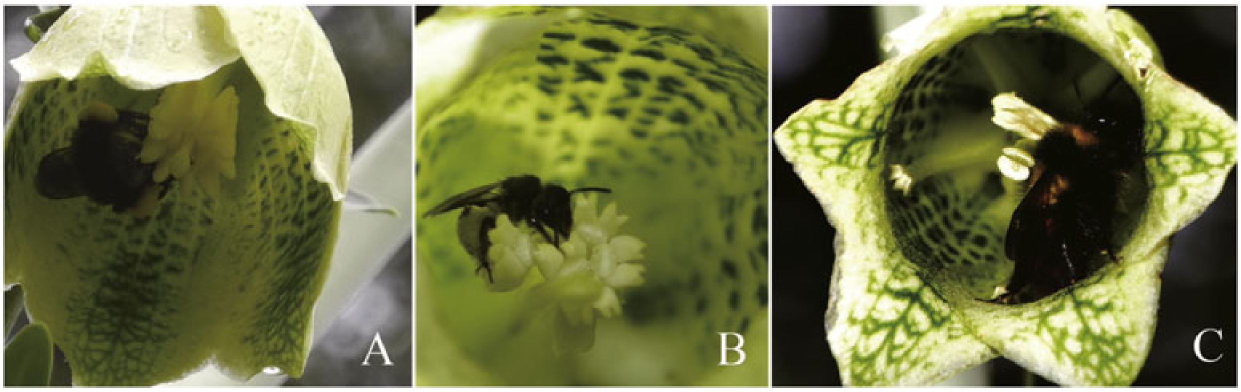

Fig. 3. Behavior of visitors on flowers of *Megacodon stylophorus*. **A,** *Bombus lucorum* collecting pollen grains from a flower in male phase. **B,** Solitary bee collecting pollen grains on a flower in male phase. **C,** *B. richardsi* taking nectar from a flower in female phase.

Table 1 Numbers of flowers (F) and possibly geitonogamously pollinated flowers (PGPF) at indicated positions on 56 monitored inflorescences of *Megacodon stylophorus*

Position	F, *n*	PGPF, *n* (%)
Apical	56	49 (87.50)
Terminal (3–5 floors)	207	138 (66.67)
Terminal (1–2 floors)	224	26 (11.61)
Middle (1–2 floors)	192	23 (11.98)

were more likely to be geitonogamously pollinated than flowers on lower floors (G test: $G = 259.32$, $d.f. = 1$, $P < 0.01$). In total, 32.90 ± 4.40% (0–10) of the monitored flowers were possibly geitonogamously pollinated through upward and horizontal pollen flow within inflorescences. Inflorescences with more flowers also had more possibly geitonogamously pollinated flowers ($R^2 = 0.69$, $n = 56$, $P < 0.001$), and the percentage of possibly geitonogamously pollinated flowers was weakly correlated with the number of total flowers ($r = 0.38$, $n = 56$, $P = 0.004$; Fig. 4).

4 Discussion

It is generally assumed that the fraction geitonogamy would significantly increase with larger display size (de Jong et al., 1993; Barrett, 2003), but the possibility of geitonogamy did not steadily increase for individuals of *Megacodon stylophorus* with more flowers. *Megacodon stylophorus* did not strictly bloom bottom-up and there were substantial frequencies of geitonogamous pollination in the monitored *M. stylophorus* population. Although there was a strong correlation between total numbers of flowers the plants produced and numbers of flowers presented on single days ($r = 0.92$), the proportion of flowers possibly geitonogamously pollinated was weakly correlated with total numbers of flowers ($r = 0.38$). This might have been because plants with more resources enlarge their floral display size mainly by producing more middle flowers on lower floors, which had a low probability of being geitonogamously pollinated (Table 1). This evidence showed that the tower-shaped inflorescences of *M. stylophorus* enlarged their size without more cost of geitonogamous mating, suggesting that natural selection favors individuals that produce extra flowers at positions within inflorescences where flowers have low probabilities of being geitonogamously pollinated.

To completely avoid geitonogamy, the optimal strategy for plants is to display a single flower per day for a long time when there is no pollinator limitation (de Jong et al., 1993; Lau et al., 2008; Karron & Mitchell, 2012). *Megacodon stylophorus* had approximately 12 flowers on average and each flower lasted approximately 8 days. Because the growth season is short in the alpine zone (Bliss, 1971), plants have a short timeframe for floral displays and often display multiple flowers simultaneously. Alternatively, geitonogamous pollination can be limited if protandrous flowers open sequentially from bottom to top and pollinators move upwards on inflorescences (Harder et al., 2004). Although the effective bumblebee pollinators largely moved upwards from lower to higher floors on inflorescences when foraging, the protandrous *M. stylophorus* did not strictly bloom bottom-up and approximately one-third of flowers per inflorescence were possibly geitonogamously pollinated through upward and horizontal pollen flow. The upward pollen flow has occurred because most apical flowers opened first in all inflorescences, and when middle flowers in lower floors began anthesis terminal flowers on the third and/or fourth floors were still in female phase. Counterpart flowers did not always bloom synchronously and even a 1-day difference in phenology might have been enough to enable horizontal pollen transfer between them (Fig. 1). We suggest the strictly bottom-up display might not always be an optimal strategy. For example, the apical flowers might be very conspicuous for bees and more important than other flowers for attracting their attention, and it is a loss if the apical flower that is most attracting for pollinators opens last. It is quite common among species possessing inflorescences with acropetal maturation that the apical flower opens first (Weberling, 1989), possibly indicating the apical flower's importance for attracting pollinators. And presenting paired branches, rather than single branches on

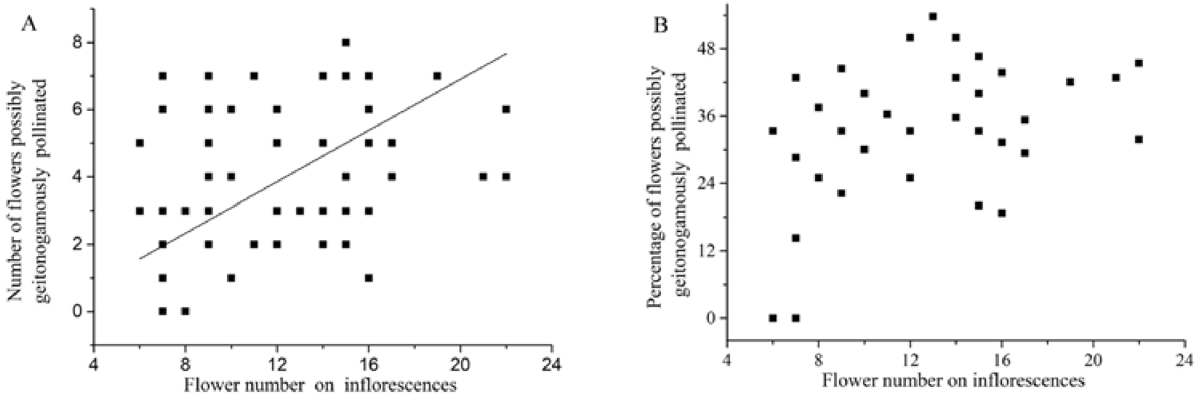

Fig. 4. **A,** Relationship between the total number of flowers on 56 monitored inflorescences of *Megacodon stylophorus* and the number of flowers possibly geitonogamous pollinated. **B,** Relationship between the total number of flowers on inflorescences and the percentage of flowers possibly geitonogamously pollinated.

each floor and producing flowers in the middle of lower branches, could save resources by shortening the floral stem and reducing the number of branches that have borne those middle flowers (Jordan et al., 2016). Further studies are needed to test these hypotheses. Actually, the evolutionary significance and the mechanisms that control flower opening coordination within inflorescences remain largely unexplored (Reeves et al., 2012; Harder & Prusinkiewicz, 2013).

It has been observed that bees typically forage upward in inflorescences since Darwin (1877), and this behavior is generally believed to enable bumblebees to maximize their net rate of nectar gain (Heinrich, 1983). Pyke (1978) suggested that the upward movement could be an adaptation to a decline in nectar volumes from lower to upper flowers. Accordingly, several studies have found a correlation between reductions in nectar volume from lower to upper flowers and upward foraging of bees (Percival & Morgan, 1965; Best & Bierzychudek, 1982; Carlson & Harms, 2006; Fisogni et al., 2011; Zhao et al., 2016). However, other studies have found that bees moved upward regardless of the distribution of nectar rewards (Heinrich, 1975; Waddington & Heinrich, 1979; Orth & Waddington, 1997). If inflorescences were visited only a few times, lower flowers might have accumulated more nectar. However, in our study, inflorescences of *M. stylophorus* were frequently visited by bumblebees and flowers in male phase produced more nectar than in female phase (Fig. 2). All these observations indicate that nectar volumes did not decline in a bottom-up pattern in the studied population of *M. stylophorus*. Heinrich (1983) suggested foraging flowers in one direction could avoid revisiting flowers when bees visit vertical inflorescences, and the upward direction is further caused by high handling efficiency than downward direction, although this hypothesis has rarely been tested.

Protandrous plants could completely avoid geitonogamous pollination while bees start foraging bottom flowers and move upwards on inflorescences by strictly presenting older and thus female flowers at the bottom of inflorescences and younger male flowers above them. However, this floral display strategy might not be optimal in the protandrous *M. stylophorus*; our observations showed that approximately one-third of flowers per inflorescence were possibly geitonogamously pollinated through upward and horizontal pollen flow. However, flowers on upper floors were more likely to be geitonogamously pollinated than flowers on lower floors, and plants with more resources enlarge their floral display size mainly by producing more flowers on lower floors, suggesting that geitonogamy might act as an important selective agent in the evolution of inflorescence architectures.

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References

Barrett SCH. 2003. Mating strategies in flowering plants: The outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society B: Biological Sciences* 358: 991–1004.

Best LS, Bierzychudek P. 1982. Pollinator foraging on foxglove (*Digitalis purpurea*): A test of a new model. *Evolution* 36: 70–79.

Bliss LC. 1971. Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics* 2: 405–438.

Carlson JE, Harms KE. 2006. The evolution of gender-biased nectar production in hermaphroditic plants. *Botanical Review* 72: 179–205.

Charlesworth D, Charlesworth B. 1987. The effect of investment in attractive structures on allocation to male and female functions in plants. *Evolution* 41: 948–968.

Darwin CR. 1877. *The various contrivances by which orchids are fertilized by insects*. London: John Murray.

de Jong TJ, Waser NM, Klinkhamer PGL. 1993. Geitonogamy: The neglected side of selfing. *Trends in Ecology and Evolution* 8: 321–325.

de Jong TJ, Waser NM, Price MV, Ring RM. 1992. Plant size, geitonogamy and seed set in *Ipomopsis aggregata*. *Oecologia* 89: 310–315.

Eckert CG. 2000. Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* 81: 532–542.

Fisogni A, Cristofolini G, Rossi M, Galloni M. 2011. Pollinator directionality as a response to nectar gradient: Promoting outcrossing while avoiding geitonogamy. *Plant Biology* 13: 848–856.

Galloway LF, Cirigliano T, Gremski K. 2002. The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. *International Journal of Plant Sciences* 163: 133–139.

Ge X-J, Zhang L-B, Yuan Y-M, Hao G, Chiang T-Y. 2005. Strong genetic differentiation of the East-Himalayan *Megacodon stylophorus* (Gentianaceae) detected by Inter-Simple Sequence Repeats (ISSR). *Biodiversity and Conservation* 14: 849–861.

Hainsworth FR, Mercier T, Wolf LL. 1983. Floral arrangements and hummingbird feeding. *Oecologia* 58: 225–229.

Harder LD, Barrett SCH. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* 373: 512–515.

Harder LD, Barrett SCH, Cole WW. 2000. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proceedings of the Royal Society B: Biological Sciences* 267: 315–320.

Harder LD, Jordan CY, Gross WE, Routley MB. 2004. Beyond floricentrism: The pollination function of inflorescences. *Plant Species Biology* 19: 137–148.

Harder LD, Prusinkiewicz P. 2013. The interplay between inflorescence development and function as the crucible of architectural diversity. *Annals of Botany* 112: 1477–1493.

Heinrich B. 1975. Energetics of pollination. *Annual Review of Ecology and Systematics* 6: 139–170.

Heinrich B. 1983. Do bumblebees forage optimally, and does it matter? *American Zoologist* 23: 273–281.

Herlihy CR, Eckert CG. 2002. Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* 416: 320–323.

Ho T-N, Pringle JS. 1995. Gentianaceae. In: Wu Z-Y, Raven PH, Hong D-Y eds. *Floral of China*. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press. 16: 1–139.

Iwata T, Nagasaki O, Ishii HS, Ushimaru A. 2012. Inflorescence architecture affects pollinator behaviour and mating success in *Spiranthes sinensis* (Orchidaceae). *New Phytologist* 193: 196–203.

Jersáková J, Johnson SD. 2007. Protandry promotes male pollination success in a moth-pollinated orchid. *Functional Ecology* 21: 496–504.

Jordan CY, Harder LD. 2006. Manipulation of bee behavior by inflorescence architecture and its consequences for plant mating. *American Naturalist* 167: 496–509.

Jordan CY, Natta M, Harder LD. 2016. Flower orientation influences the consistency of bumblebee movement within inflorescences. *Annals of Botany* 118: 523–527.

Karron JD, Mitchell RJ. 2012. Effects of floral display size on male and female reproductive success in *Mimulus ringens*. *Annals of Botany* 109: 563–570.

Klinkhamer PGL, de Jong TJ. 1993. Attractiveness to pollinators: A plant's dilemma. *Oikos* 66: 180–184.

Lau JA, Miller RE, Rausher MD. 2008. Selection through male function favors smaller floral display size in the common morning glory *Ipomoea purpurea* (Convolvulaceae). *American Naturalist* 172: 63–74.

Liao W-J, Harder LD. 2014. Consequences of multiple inflorescences and clonality for pollinator behavior and plant mating. *American Naturalist* 184: 580–592.

Meng L-H, Wang Y, Luo J, Liu C-Y, Yang Y-P, Duan Y-W. 2012. Pollination ecology and its implication for conservation of an endangered perennial herb native to the East-Himalaya, *Megacodon stylophorus* (Gentianaceae). *Plant Ecology and Evolution* 145: 356–362.

Orth AI, Waddington KD. 1997. The movement patterns of carpenter bees *Xylocopa micans* and bumblebees *Bombus pennsylvanicus* on *Pontederia cordata* inflorescences. *Journal of Insect Behavior* 10: 79–86.

Percival M, Morgan P. 1965. Observations on the floral biology of *Digitalis* species. *New Phytologist* 64: 1–22.

Pyke GH. 1978. Optimal foraging: Movement patterns of bumblebees between inflorescences. *Theoretical Population Biology* 13: 72–98.

Rademaker MCJ, De Jong TJ, Van Der Meijden E. 1999. Selfing rates in natural populations of *Echium vulgare*: A combined empirical and model approach. *Functional Ecology* 13: 828–837.

Reeves PH, Ellis CM, Ploense SE, Wu M-F, Yadav V, Tholl D, Chételat A, Haupt I, Kennerley BJ, Hodgens C, Farmer EE, Nagpal P, Reed JW. 2012. A regulatory network for coordinated flower maturation. *PLoS Genetics* 8: e1002506.

Routley MB, Husband BC. 2003. The effect of protandry on siring success in *Chamerion angustifolium* (Onagraceae) with different inflorescence sizes. *Evolution* 57: 240–248.

Snow AA, Spira TP, Simpson R, Klips RA. 1996. The ecology of geitonogamous pollination. In: Lloyd DG, Barrett SCH eds. *Floral biology*. New York: Chapman and Hall. 191–216.

Utelli AB, Roy BA. 2000. Pollinator abundance and behavior on *Aconitum lycoctonum* (Ranunculaceae): An analysis of the quantity and quality components of pollination. *Oikos* 89: 461–470.

Waddington KD, Heinrich B. 1979. The foraging movements of bumblebees on vertical "inflorescences": An experimental analysis. *Journal of Comparative Physiology* 134: 113–117.

Weberling F. 1989. *Morphology of flowers and inflorescences*. Cambridge: Cambridge University Press.

Zhao Z, Lu N, Conner JK. 2016. Adaptive pattern of nectar volume within inflorescences: Bumblebee foraging behavior and pollinator-mediated natural selection. *Scientific Reports* 6: 34499.