Reproductive ecology of the Qinghai-Tibet Plateau endemic
*Gentiana straminea* (Gentianaceae), a hermaphrodite perennial
characterized by herkogamy and dichogamy

Yuan-wen Duan a,c,1, Ya-ping He a,1, Jian-quan Liu a,b,*

**a** Qinghai-Tibet Plateau Biological Evolution and Adaptation Laboratory, Northwest Institute of Plateau Biology, the Chinese Academy of Sciences, Xining 810001, P. R. China

**b** Key Laboratory of Arid and Grassland Ecology, Lanzhou University, Lanzhou 730000, P. R. China

**c** Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China

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**Abstract**

The combined occurrence of both herkogamy and dichogamy in a hermaphrodite species has been considered to strongly favour outcrossing. In this study, we investigated in detail the reproductive ecology of *Gentiana straminea* Maxim. (Gentianaceae), a hermaphrodite perennial endemic to the Qinghai-Tibet Plateau. In a series of observations and experiments over four consecutive years, we examined whether the combination of dichogamy and herkogamy in individual flowers completely prevents geitonogamous pollen transfer in this species. The mode of floral development clearly indicates that autonomous self-pollination is completely avoided through herkogamy and dichogamy in individual flowers. This implication was confirmed by the breeding experiments, since no seed was produced when flowers were isolated. However, this gentian proved to be highly self-compatible when geitonogamous selfing was artificially induced. Many flowers opened simultaneously on individual plants, the ratio of male to female phase flowers was close to 2:1 in each inflorescence at the full anthesis phase, and they were randomly distributed amongst the upper, middle and lower parts of each stem’s inflorescence. On average, *Bombus sushikini* Skorikov, the most frequent visitor and only legitimate pollinator of *G. straminea*, visited nearly two flowers per inflorescence, and four flowers per plant. Among the pollinators’ foraging bouts, the proportions of geitonogamous visits to inflorescences or flowers within an individual plant were 29% and 37%, respectively. Therefore, despite the strict dichogamous and herkogamous characteristics of the individual flowers, geitonogamous selfing might still prevail in *G. straminea* because of the size of its floral displays and the continuous visiting behavior of *B. sushkini*.

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**Keywords:** Breeding system; Floral display; Geitonogamy; *Bombus sushkini*

1. Introduction

Dichogamy and herkogamy, two common features of flowering plants, are suites of characteristics that promote temporal and spatial separation of pollen presentation and receipt within flowers, respectively. A combination of these two floral mechanisms greatly reduces intra-flower self-pollination and minimises bisexual interference, thereby providing an effective mechanism for promoting outcrossing and reducing self-pollination (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Barrett, 1998, 2002). However, dichogamy, herkogamy or a combination of these characteristics may exclude the possibility of geitonogamous pollen transfer, depending on floral architecture, flowering sequence, pollinator behaviour and visiting frequency. For example, if female-phase flowers are situated below male-phase flowers on an irregular inflorescence, upward foraging pollinators, such as bumblebees, this may reduce the potential for geitonogamous pollen transfer (Darwin, 1876; Best and Bierzychudek, 1982). In contrast, if pollinators forage downwards on this type of inflorescence, selfing is likely to occur. Pollinator behaviour is clearly critical in such cases. In a study of *Campanula americana* L. by
Galloway et al. (2002), the ratio of flowers in male and female phases did not affect bee behaviour, and the bees did not show a gender preference. Thus, the occurrence of flowers in female phase below those in male phase on reproductive branches did not reduce geitonogamy in this species. In addition, increase in floral display size will positively influence such geitonogamous pollen transfer (De Jong et al., 1993; Snow et al., 1996; Ishii and Sakai, 2002). In contrast, the effects of floral display size on geitonogamous pollination in *Northcicum asiaticum* Maxim. should theoretically be minor as most pollinators leave a raceme after visiting one flower, irrespective of the raceme display size (Ishii and Sakai, 2002). However, inflorescence architecture is less structured in many dichogamous species (Rademaker et al., 1999).

Little is known of dichogamy and herkogamy on the exclusion of pollen transfer between flowers within individuals in such types of loosely structured or unstructured inflorescences. In Gentianaceae, herkogamy and dichogamy, singly or together, have been reported for some species (Webb and Littleton 1987; Petanidou et al., 2001; Petanidou et al., 1991, 1995; Petanidou et al., 1998; Bynum and Smith, 2001). Webb and Littleton (1987) speculated that geitonogamy may occur in *Gentiana serotina* Cockayne and *G. saxosa* Forster, since many flowers are found on individual plants of these species, presenting pollen or stigma simultaneously and randomly in spite of their distinct protandrous dichogamy. However, such speculation has never been tested in the field. The distribution of gentians is centred in the mountains of Southwest China, particularly the eastern Qinghai-Tibet Plateau. Although more than two thirds of all species of the family are found here, most of which are endemic to this area (Ho and Liu, 2001), all previously studied species with records of herkogamy and dichogamy originate beyond this area. In addition, the inflorescences of most perennial gentians distributed there are loosely structured (Ho and Liu, 2001). If herkogamy and dichogamy prevail, they are ideal models for studying the effects of the two mechanisms on mating patterns for species with unstructured inflorescences. Consequently, we observed the floral ecology and pollination biology of the endemic and abundant *G. straminea* Maxim. in one population across 4 years on the Qinghai-Tibet Plateau. Studies in which pollinators have been observed and mating patterns directly measured have demonstrated that geitonogamy increases the selfing rate (Vrieling et al., 1999), reduces the outcrossing rate (Harder and Barrett, 1995) and that within-plant pollinator movement may lead to geitonogamous selfing. In the course of the studies, we specifically address the following question: can a combination of herkogamy and dichogamy effectively avoid geitonogamous pollen transfer and selfing in gentians with unstructured inflorescences?

2. Materials and methods

2.1. Study site and species

This study was carried out between late July and September from 2000 to 2003. The population we studied is located at the Haibei Alpine Meadow Ecosystem Research Station of the Chinese Academy of Sciences on the northeast Qinghai-Tibet Plateau (lat. 37°29′–37°45′ N, long. 101°12′–101°23′ E, alt. 3200 m). The average annual air temperature is ~1.7 °C with extremes of 27.6 °C (maximum) and ~37.1 °C (minimum). Annual precipitation ranges between 426 and 860 mm, 80% of which falls in the short summer growing season from May to September but mainly in July and August. The study area is dominated by *Kobresia humilis* (C. A. Mey.) Serg., while *G. straminea* occurs as a subdominant herb. *G. straminea* flowered from mid-July in 2000, late July in 2001 and 2002, and early August in 2003. In this community, *Aster diplostephoides* (DC.) C. B. Clarke, *Pedicularis kansuensis* Maxim., *Aconitum gynanandrum* Maxim., *Oxypolis ochrocephala* Bunge. and *Delphinium caeruleum* Jacq. ex Camb. flower at the same time as *G. straminea*.

*G. straminea* is a herbaceous perennial of alpine environments and high mountains of the Qinghai-Tibet Plateau in China, and thrives at altitudes between 2600 and 4600 m (Ho and Liu, 2001). The individual flowers are 2–4 cm in length and have an erect, funnel-shaped corolla comprising five connate petals. The gynoecium comprises a single bircarpellate ovary with parietal placentation bearing numerous lines of ovules. Five floral nectaries are situated around the base of the ovary.

2.2. Experimental design

**Herkogamy and dichogamy**- To test for the presence of herkogamy in *G. straminea*, one flower in the male phase and one in the female phase were randomly chosen from each of 98 individual plants at least 5 m apart. The heights of the androecium and gynoecium in the male- and female-phase flowers were measured separately using vernier callipers. To examine the dichogamous processes and longevity of individual flowers in natural condition, in each of the three years, we selected 70 floral buds from 10 random plants. Floral development was divided into four phases and monitored: (1) bud phase (pre-dehiscence of the corolla); (2) male phase (from the commencement of flower opening to initial stigma lobe opening); (3) female phase (from the initial stigma lobes opening to permanent closure) and (4) fruiting phase (after permanent closure, but prior to fruit dehiscence).

**Breeding system**- In each of the four years, we subjected *G. straminea* flowers to the following treatments: (1) control; (2) emasculation without isolation; (3) isolation without emasculation; (4) isolation after emasculation; (5) artificial geitonogamy (emasculation followed by artificial pollination with fresh pollen from the same plants); and (6) artificial xenogamy (emasculation followed by pollination with fresh pollen from different plants). Treatments (1) and (3) were carried out at bud phase while emasculation in treatment (2), (4), (5) and (6) was done before stigma lobe opened, and we used paper bags for isolation, in which there was enough space for flower opening and closing. All fruits were collected before dehiscence to count seed sets. Because the ovules vary greatly
in the different flowers, we used the seed set ratio, calculated by mature seeds divided by the total ovules in each ovary, to assess the breeding results in each treatment.

**Pollinators and floral display** - In the full anthesis phase in the years 2000–2003, the visitors to G. straminea were monitored for 3–6 days, respectively, and the total observation time amounted to more than 150 h. In the field, the behaviour and visiting frequency of each species of taxa were recorded using methods described by Arroyo et al. (1985). Visitors were identified in Institute of Zoology, the Chinese Academy of Sciences. We also stochastically selected representative visitors of each species captured on flowers of G. straminea to examine for pollen existence using a Hitachi S-800 SEM. The pollination effectiveness of bumblebees was evaluated based on the seed set of the once-pollination for the isolated flowers with emasculation. In this treat, the flowers were firstly emasculated and isolated by the paper bags. At the female stage, we removed the paper bags and waited for the pollination by bumblebees. Once pollinated once, the flower was isolated ago.

To investigate the influence of the behaviour of frequent, legitimate pollinators and that of floral display size on the reproductive biology of G. straminea, flower numbers and visitor numbers were recorded during full anthesis. Pollinator behaviour and flower numbers visited were recorded in 2002 and 2003. We observed the behaviour of 42 Bombus sushikini Skorikov pollinators over a 10-day period and recorded the flower numbers of 1111 visited inflorescences, in which we recorded number of visited flowers on 641 inflorescences. We followed these bumblebees to monitor their movements between flowers within an inflorescence, or between inflorescences within an individual plant or between individual plants. The proportions of potentially geitonogamous (movements between flowers within an inflorescence or between inflorescences within an individual plant) and xenogamous (movements between different individual plants) visits were calculated from continuous foraging bouts of 20 legitimate pollinators only to individuals of G. straminea. The remaining 22 bouts were only found to visit one G. straminea plant, and then left for other species and these data can be added to compute the potentially geitonogamous visits (with more than one flower within one plant). We also recorded the number and locations of the male- and female- phases flowers on 892 inflorescences at full anthesis in 2000, 2001 and 2002. These data were used to calculate six variables: (1) numbers of flowers in the male and female phase per inflorescence, (2) distribution frequency of opening flower numbers, (3) flower numbers and proportions visited for inflorescences of different sizes, (4) proportions of geitonogamous and xenogamous visits. In addition, we recorded the numbers of male- and female-phase flowers probed by pollinators. Due to the disturbance to pollinator activity caused by our investigations, only 10 such inflorescences were successfully observed. Additionally, we captured the most frequent and legitimate visitor and measured their tongue length when our observations were completed. We also measured the depth of the corolla to examine the correspondence with the tongue length of visitor.

### 2.3. Statistical analyses

One-way ANOVA and Post hoc-LSD analysis was used to examine differences in androecium and gynoecium height in different years in male- and female-phase flowers, as well as differences in sexual longevities in through phenological phases and seed sets associated with different treatments over the experimental period. Flower numbers in male and female phases in each inflorescence were examined using Paired Sample T tests and Paired Sample correlations. Pearson correlations were used to examine difference between inflorescence size-frequency distribution for (a) inflorescences in natural populations and (b) those observed when recording the pollinators’ behaviour or the number of flowers and size of inflorescences that pollinators visited. Prior to comparison, the data were tested for normality. Data analysis was performed on and figures generated using SPSS ver. 11.0. Here, and below, the figures are means ± 1SE and N refers to the sample size.

### 3. Results

**Dichogamy and herkogamy** - In the bud phase, the sepals envelope the corolla, which elongates and spreads prior to opening. When a flower begins to open, the anthers have already dehisced, thus presenting pollen near the centre of the flower, and the stigma lobes, sited lower than the anthers, are tightly closed. This condition lasted for 3.0 ± 0.2 days in 2000 (N = 30), 5.2 ± 0.2 days in 2001 (N = 20), 3.7 ± 0.1 days in 2002 (N = 27), and 7.1 ± 0.1 days in 2003 (N = 18) (means are presented as ± 1SE). In the late male phase, the androecium moved outwards toward the corolla, and finally leaned against it. Simultaneously, the stigma elongated and opened its lobes about a day later. The height of the androecium showed no significant difference either between male and female phases or among the three years (Fig. 1), but the stigmas were clearly longer in the female phase than in the male phase (Fig. 2). The female phase lasted 5.1 ± 0.4 days in 2000 (N = 31), 4.5 ± 0.3 days in 2001 (N = 19), 4.5 ± 0.2 days in 2002 (N = 20), and 5.5 ± 0.2 days in 2003 (N = 18).

Two days after pollination, the corolla lobes closed imbricately, forming a compact structure similar to, but slightly larger than a late bud. The corolla slowly turned brown and withered as the capsule reached maturity after about four weeks. Both male longevity and individual flower longevity varied greatly between the four years, but female longevity was relatively stable.

**Breeding system** - Whether they were emasculated or not, none of the 300 isolated G. straminea flowers examined over the four years period produced any seed, indicating that no apomixis or autonomous self-pollination occurs in this species. However, the high seed set ratio of geitonogamous fruits
produced over the period show that *G. straminea* is self-compatible (Table 1). The differences between seed set ratio of artificially pollinated flowers and control differed significantly in 2000 and 2001, but not in 2002 and 2003, suggesting that there is no pollen limitation in natural seed production of *G. straminea*. Seed set ratio for each treatment varied between years, except for treatment of emasculation without isolation (Table 1).

**Floral display and flower sequence**—*G. straminea* has a well-developed basal vegetative rosette with a terminal bud growing continuously throughout the life of the plant. The annual flowering stems grow out from the axillary buds of the outer leaves of the basal rosette. Individual plants have 1–6 stems (mean 2.1 ± 0.1, *N* = 153) and each stem bears 1–18 flowers (7.5 ± 0.2, *N* = 321) in both terminal and axillary cymes with flowers in the male phase outnumbered by those in the female phase (Fig. 3, Fig. 4: C). Usually, the terminal flower on the cymes opened first, but the flowering sequence of the inflorescences on each stem did not follow any clear pattern. In the full anthesis phase during our observations, individual plants displayed 7.0 ± 0.4 (*N* = 154) flowers: 5.6 ± 0.4 (*N* = 154) in the male phase and 1.4 ± 0.1 (*N* = 154) in the female phase.

### Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Control</th>
<th>Emasculation without isolation</th>
<th>Artificial geitonogamy</th>
<th>Artificial xenogamy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>0.6 ± 0.0002 (46)</td>
<td>–</td>
<td>0.8 ± 0.0001 (16)</td>
<td>0.8 ± 0.0001 (65)</td>
</tr>
<tr>
<td>2001</td>
<td>0.6 ± 0.0002 (91)</td>
<td>0.7 ± 0.1892 (15)</td>
<td>0.7 ± 0.0001 (26)</td>
<td>0.6 ± 0.0001 (17)</td>
</tr>
<tr>
<td>2002</td>
<td>0.8 ± 0.0001 (11)</td>
<td>0.6 ± 0.1892 (6)</td>
<td>0.7 ± 0.0001 (16)</td>
<td>0.7 ± 0.0001 (28)</td>
</tr>
<tr>
<td>2003</td>
<td>0.6 ± 0.1892 (16)</td>
<td>0.5 ± 0.1892 (8)</td>
<td>0.8 ± 0.0001 (17)</td>
<td>0.8 ± 0.0001 (20)</td>
</tr>
</tbody>
</table>

Values with the same superscript letter in the same column, or the same superscript number in the same row, do not differ significantly at the 0.05 level. Seed set ratio values for treatment of emasculation without isolation in 2000 were unavailable because the plants were destroyed by yaks. Numbers in bracket mean the sample size.
Visitors and Pollinators- During the four years’ observation period, 14 species belonging to two classes, seven orders and eight families visited to *G. straminea*; these included four species of bumblebee, one species of bee, two species each of flies and ants, and one species each of thrips, beetles, spiders, ladybugs and stink bugs (except for thrips and bees, we failed to identify species name of the remaining insects because of unavailable taxonomists). According to the visiting behaviour of these taxa, *B. sushikini* appears to be the most consistent and effective pollinator of *G. straminea* (Fig. 4: A). Its large body size contacted both stigma and anthers effectively. A large number of *B. sushikini* were observed to visit *G. straminea* each year with a high visit frequency to the marked flowers (average 0.005 times minute$^{-1}$ flower$^{-1}$ from 10 am in the morning to 6 pm in the afternoon, the total monitoring hours: 2001, 24 h; and 2002, 390 h). All captured individuals (*N* = 15, 2000; *N* = 20, 2001; *N* = 12, 2002; *N* = 18, 2003) on *G. straminea* were found to carry a large number of pollens using nude eyes and almost all of these pollen grains on captured *B. sushikini* (Fig. 4: E, F) (*N* = 3) were identified as *G. straminea* under SEM (Fig. 4: D), and the once-pollinated flowers (*N* = 17) by this bumblebee produced a high seed set ratio (0.2 ± 0.0). However, the honeybees, *Apis mellifera* L., also frequent visitors, visited only male-phase flowers of *G. straminea* for pollen (Fig. 4: B) and did not visit female phase or the artificially emasculated flowers. Other taxa are not effective pollinators of *G. straminea*, partly due to the low visiting frequency, and partly due to the incompatibility between visitor and flower morphology because they rarely made effective contact with the anthers or stigmas since their size and shape were inappropriate for the relatively large flowers. *G. straminea* pollen was rarely observed on these insects under SEM except for honeybees and thrips. Another bumblebee species, *Bombus kashmirensis* Friese, also visited *G. straminea*, but only probed the corolla base for nectar from outside without touching the anthers or stigmas. Although two other bumblebee species, *Bombus flinchera* Vogt and *Bombus woltoni* Ckll, also might show similar visiting behavior and pollination effectiveness to *B. sushikini*, their visit rates were very low: only one *B. flinchera* was observed in each of the two years 2000 and 2002, and one *B. woltoni* in 2001 in all hours monitored during the four-year study period. Additionally, the tongue length (13.7 ± 3.3 mm, *N* = 25) of *B. sushikini* corresponds with the depth of the corolla tube (13.5 ± 1.2 mm, *N* = 35) of *G. straminea* for foraging nectar (*F* = 0.115, df = 1, *P* = 0.736). During foraging bouts, *B. sushikini* maintained high fidelity to flowers of *G. straminea* irrespective of flower gender phase despite that they also visited other co-flowering species, e.g. *P. kansuensis*. *B. sushikini* visited 1.7 ± 0.8 female- and 0.9 ± 0.8 male-phase flowers of each inflorescence, with the proportions of downward and upwards visits being approximately 1:1 (*N* = 10). Following movements of 20 *B. sushikini* bumblebees, which continuously visited *G. straminea* plants, among the visited 361 inflorescences and 596 flowers, 103 (29%) and 215 (37%), respectively, were restricted to single plants (Table 2). In addition to the remaining records of 22 bouts, which were restricted only to one plant, *B. sushikini* visited 1.8 ± 1.0 (*N* = 641) flowers per inflorescence, with 48% of visits being made to more than one flower per inflorescence. With an average of two inflorescences per plant, the number of flower visited per plant was approximately 3.6 ± 1.0. The larger open floral display received a greater number of visits (Pearson correlation: *R* = 0.993, *P* < 0.001).

### 4. Discussion

A combination of dichogamy and herkogamy hinders autonomous and facilitated self-pollination by separating pollen removal and deposition into two distinct phases both temporally and spatially (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Barrett, 1998, 2002). In addition, the combination of dichogamy and herkogamy helps minimise sexual interference during pollination (irrespective of vector). In hermaphrodite plants, flowers receive and disperse pollen, and thus function as both maternal and paternal parents to the next generation of individuals (Barrett, 2002). These dual sex roles undoubtedly cause conflicts in parental roles during pollination and mating (Fetscher, 2001; Barrett, 2002). Floral features that promote the temporal and spatial separation of male and female functions, e.g. dichogamy and herkogamy, etc., can greatly alleviate such conflicts in sexual roles (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Barrett, 1998, 2002).

In the first male phase, in *G. straminea*, anthers occupied the flower centre and the stigma lobe was closed. The duration of pollen shedding varied greatly. Such variation might be consequence of variation in the activity of pollen vectors over different years, for rapid removal of pollen may shorten the duration of the male phase (Lloyd and Barrett, 1996). In the following female phase, anthers without pollen moved to the corolla, while the stigma (with open lobes) elongated and replaced them. The movement of the androecium and elongation of the gynoecium at the appropriate time facilitated the completion of the temporal and spatial separation of the

### Table 2

<table>
<thead>
<tr>
<th>Open flowers number per inflorescence</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visited inflorescence number</td>
<td>108</td>
<td>148</td>
<td>160</td>
<td>135</td>
<td>53</td>
<td>26</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Proportion of visited inflorescences (%)</td>
<td>0.17</td>
<td>0.24</td>
<td>0.25</td>
<td>0.21</td>
<td>0.25</td>
<td>0.21</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Visited flower number per inflorescence</td>
<td>1.0 ± 0.0</td>
<td>1.4 ± 0.6</td>
<td>1.8 ± 0.8</td>
<td>2.2 ± 1.1</td>
<td>2.4 ± 1.1</td>
<td>2.8 ± 1.6</td>
<td>2.8 ± 1.3</td>
<td>3.7 ± 1.5</td>
<td>5.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Correlation coefficient between visited flower number per inflorescence and open flowers number per inflorescence was 0.993 (*P* < 0.001, Pearson correlation).
male and female phases both functionally and morphologically. A similar combination of dichogamy and herkogamy has been recorded in *Gentiana newherryi* Gray (Spira and Pollak, 1986), *Gentiana pneumonanthe* L. (Petanidou et al., 2001), *Gentiana cruciata* L. (Petanidou et al., 1991, 1995), and *G. algida* Pallas (Bynum and Smith, 2001). However, in *Gentianella germanica* (Wild) Börner and *Gentianella campestris*, only herkogamy has been found (Petanidou et al., 1998, references therein), in *Gentiana serotina* and *Gentiana saxosa* Forster f. only dichogamy (Webb and Littleton 1987), and neither occurs in *Gentiana saxosa* Forster f. only dichogamy (Webb and Littleton 1987), and neither occurs in *Gentianella uliginosa* (Wild) Börner, resulting in autonomous selfing (Petanidou et al., 1998).

Both previous studies and our investigation indicate that the occurrence of dichogamy and herkogamy in the development of individual flowers would appear to be a common phenomenon in gentians, especially the genus *Gentiana*. During the flowering period of *G. straminea*, strongly protandrous dichogamy occurs in *G. straminea*. Furthermore, there were no significant differences in androecium height between male and female phases or over the 3 years they were measured (Fig. 1), while heights of the gynoecium in male and female phases were clearly different (Fig. 2), suggesting herkogamy.

The mode of floral development would indicate that autonomous self-pollination is completely avoided through herkogamy and dichogamy in individual flowers of *G. straminea*. This conclusion was further confirmed by breeding experiments during the four years of the study, since no seed was produced when flowers were isolated. The main pollinator of this gentian was found to be *B. sushikini*. This finding is consistent with pollination research on other gentians, in which bumblebees were found to be consistent and effective pollinators (Petanidou et al., 1991, 1995, 2001; Bynum and Smith, 2001).

However, even together, dichogamy and herkogamy may not completely prevent geitonogamous selfing in *G. straminea* due to the following: (1) *G. straminea* is highly self-compatible, allowing geitonogamous fertilization to occur within the plant. (2) The flowering sequence in this species is irregular, with male and female flowers randomly distributed amongst the upper, middle and lower parts of the stem. (3) Most individuals have more than one stem. (4) *B. sushikini* visit approximately four flowers per plant. Although flowers in the male phase outnumbered those in the female phase during the full anthesis phase (Fig. 3), bumblebees consistently approached female flowers nearly twice as often as male flowers. In addition, they generally moved upwards or downwards along inflorescences. The quantitative records of geitonogamous visits indicate that 29% of movements occurred between inflorescences, and 37% between flowers within an individual plant. Therefore, geitonogamous pollen transfer could not be ruled out, despite the strong dichogamy and herkogamy observed in the development of individual flowers.

A number of studies have demonstrated that floral display size also affects the amount of geitonogamous pollination and, in turn, the degree of both pollen discounting and self-pollination (De Jong et al., 1993). One effect that display size can have on geitonogamy is associated with changes in the gender ratio of the flowers (Sarkissian et al., 2001). The proportion of female flowers increases with plant size in two monoeocious *Sagittaria* species, and the possibility of geitonogamy will increase greatly if the plants are self-compatible and flowers in male and female phases bloom at the same time (Huang et al., 2002; Sarkissian et al., 2001). However, the ratio of flowers in male and female phases did not vary with floral display size in *G. straminea* (Fig. 3). On average, plants had almost twice as many flowers in the male phase compared to the female phase. The constant ratio of flowers in the male and female phases, irrespective of inflorescence size, is not consistent with previous findings in hermaphrodite species, such as *Campanula americana* (Galloway et al., 2002). The main effect of floral display size upon geitonogamy is to change the visit frequency of pollinators to an individual plant. In *Narthecium asiaticum*, such effects of display size on geitonogamous pollination would be minor, as most pollinators leave a raceme after visiting one flower, irrespective of display size (Ishii and Sakai, 2002). In contrast, pollinators of *G. straminea* frequently and continuously visited several flowers of a single stem inflorescence or the same plant: geitonogamous visits increased with the size of the inflorescences (Pearson correlation: $R = 0.993$, $P < 0.01$). For a given display size, selfing probably occurs more often in isolated plants (Mustajarvi et al., 2001; Galloway et al., 2002). Therefore, the display size of flowers and the density of plants in naturally distributed populations may be major determinants in the degree of geitonogamy in *G. straminea*, but this area requires further research (Table 2).

It has been suggested that geitonogamy may be avoided in *Butomus umbellatus* L. because dichogamy is synchronous across flowers across all inflorescences of this species. Bhardwaj and Eckert (2001), however, found that the directions of pollinators’ visits were irregular, and that visitors usually foraged in more than one flower per umbel. Geitonogamy cannot be avoided, however, if synchronous dichogamy is incomplete (Davila and Wardle, 2002). For flowering species that are asynchronously dichogamous, avoidance of geitonogamy would occur only if pollinators visited female-phase flower before male-phase or visited only one flower per individual plant. Galloway et al. (2002) reported that pollinators usually visited male flowers before female-phase flowers of *C. americana*. This species has a loosely structured inflorescence, and the number of flowers in the female phase visited after one or more flowers in the male phase increased with floral display size, resulting in geitonogamy. Even in species with regular inflorescences, pollinators moving between different inflorescences within an individual plant would undoubtedly result in geitonogamous pollen transfer, e.g. in self-compatible populations of *Aconitum lycoctonum* s.l. (Utelli and Roy, 2000). In species with unstructured inflorescences (e.g. *G. straminea*), geitonogamy can only be avoided when pollinators visit one flower per individual plant in each foraging bout. However, such cases are rarely found in *G. straminea*. 

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and the other reported species. Nevertheless, we tentatively suggest that dichogamy and/or herkogamy play an important role in this species by reducing interference between male and female functions (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Barrett, 1998, 2002), as well as favouring out-crossing and reducing geitonogamous selfing.

Because the present study was conducted based on only one population of G. straminea although continuing for 4 years, it remains necessary to investigate whether these findings prevail in the other populations of this species. However, G. straminea occurs mainly in the alpine meadow of the Qinghai-Tibet Plateau (Ho and Liu, 2001) and most of the remaining distributions in the plateau have a similar precipitation, climate, soil and vegetation dominated by Kobresia species (Wu, 1980). Our ongoing researches of the other two populations nearly 100 km away from the present study one revealed similar results and conclusions as those presented here (e.g. the same pollinators, similar visit frequencies and unavoidable geitonogamy, Duan et al., unpublished). In the recent years, this medically important species had continuously been collected and the size of natural populations decreased drastically (Ho and Liu, 2001). The unavoidable geitonogamous selfing found in this species may further create possible inbreeding depression, and deteriorate its restoration and conservation in the future. We found no evidence for inbreeding depression at the seed set stage, since no significant difference was found between artificial geitonogamous pollination and artificial xenogamy during 4 years (Table 1). However, inbreeding depression often occurs late in the life cycle of plants (Husband and Schemske, 1996; Dudash and Fenster, 2001), and whether and how much the inbreeding depression will affect offspring fitness of G. straminea needs to be further elucidated in the future researches.

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