Effective pollinators of Asian sacred lotus (*Nelumbo nucifera*): contemporary pollinators may not reflect the historical pollination syndrome

Jiao-Kun Li and Shuang-Quan Huang*

College of Life Sciences, Wuhan University, Wuhan 430072, China

Received: 28 March 2009 Returned for revision: 7 May 2009 Accepted: 3 June 2009 Published electronically: 17 July 2009

**Background and Aims** If stabilizing selection by pollinators is a prerequisite for pollinator-mediated floral evolution, spatiotemporal variation in the pollinator assemblage may confuse the plant–pollinator interaction in a given species. Here, effective pollinators in a living fossil plant *Nelumbo nucifera* (Nelumbonaceae) were examined to test whether beetles are major pollinators as predicted by its pollination syndrome.

**Methods** Pollinators of *N. nucifera* were investigated in 11 wild populations and one cultivated population, and pollination experiments were conducted to examine the pollinating role of two major pollinators (bees and beetles) in three populations.

**Key Results** Lotus flowers are protogynous, bowl shaped and without nectar. The fragrant flowers can be self-heating during anthesis and produce around 1 million pollen grains per flower. It was found that bees and flies were the most frequent flower visitors in wild populations, contributing on average 87.9 and 49.4% of seed set in Mishan and Lantian, respectively. Beetles were only found in one wild population and in the cultivated population, but the pollinator exclusion experiments showed that beetles were effective pollinators of Asian sacred lotus.

**Conclusions** This study indicated that in their pollinating role, beetles, probable pollinators for this thermoregulating plant, had been replaced by some generalist insects in the wild. This finding implies that contemporary pollinators may not reflect the pollination syndrome.

**Key words:** *Nelumbo nucifera*, beetle pollinated, pollination syndrome, effective pollinator, generalized flower, thermoregulation, Nelumbonaceae.

**INTRODUCTION**

The evolution of floral traits has been thought to be moulded by their most frequent and effective pollinators (Stebbins, 1970). To achieve pollination, animal-pollinated flowers have evolved suites of convergent floral traits, namely flower shape, flower colour, flower scent and type of reward or floral herbivory defence, appropriate to the foraging preferences and morphology of their particular pollinators, forming a specialized relationship between plants and pollinators (Grant and Grant, 1965; Barker and Hurd, 1968; Stebbins, 1970; Fægri and van der Pijl, 1979; Crepet, 1983; Pellmyr, 2002). The concept of pollination syndromes has been a fundamental subject in plant reproductive ecology because of its capacity to predict the type of pollinators of a given species based on observations of certain floral traits (Pellmyr, 2002; Fenster *et al.*, 2004). Specialized pollination systems are rare, however, and the reliability of pollination syndromes has been questioned recently (Erhardt, 1993; Fishbein and Venable, 1996; Waser *et al.*, 1996; Lippok and Renner, 1997; Ollerton and Liebe, 1997; Johnson and Steiner, 2000; Waser and Ollerton, 2006). Many plants with specialized floral traits are often visited by several types of pollinators and in some cases the primary pollinators predicted by pollination syndromes play only minor roles in reproductive success (Waser, 1979; Wilson and Thomson, 1991; Fleming *et al.*, 2001; Mayfield *et al.*, 2001; Rivera-Marchand and Ackerman, 2006). Therefore, to quantify the role of pollinators in moulding floral traits, there is a need to investigate not only the specific pollinators predicted by the pollination syndrome, but also the contributions of multiple other pollinators to plant reproductive success.

Asian sacred lotus *Nelumbo nucifera* Gaertn (Nelumbonaceae) is one of the basal angiosperms, a living fossil occurring in the Old World (Borsch and Barthlott, 1994). The lotus flower is bowl shaped and nectarless with an obvious cantharophily syndrome (i.e. beetle pollinated), having a large chamber enclosed by petals and a large number of stamens, and releasing odours. The flower can physiologically regulate the floral temperature during anthesis by self-heating via a large obconical receptacle, maintaining a relatively favourable temperature between 30 and 36°C when the surrounding temperature fluctuates between 10 and 45°C (Seymour and Schultze-Motel, 1996, 1998; Seymour *et al.*, 1998). This homoeothermic flower has been considered as a warm night shelter for beetle activity (Seymour *et al.*, 2003) and a type of beetle-pollinated chamber blossom (Vogel and Hadacek, 2004; Davis *et al.*, 2008). The lotus flower closes during the night by petal movement, but it is accessible to diverse insects because it opens by day and provides a large pollen reward. Although the effective pollinators in the Asian lotus remain unknown (Vogel and Hadacek, 2004), a study on the American lotus *Nelumbo lutea* (syn. *N. pentapetala*) indicated that bees as well as beetles are important pollinators in some...
populations (Sohmer and Sefton, 1978; Schneider and Buchanan, 1980). However, beetles were not entrapped in the first-night flowers of *N. lutea*, which otherwise facilitates outcross pollination (Schneider and Buchanan, 1980), and beetles were not present throughout the flowering period and were considered to be poor pollen vectors (Bernhardt, 2000), throwing doubt on the role of thermoregulation and cantharophily in lotus flowers.

Studies supporting the prediction of pollination syndromes usually involve species with specialized flowers, such as tubular flowers with nectar, often employing a group of effective pollinators (Castellanos et al., 2003; Wilson et al., 2004; Muchhala, 2006; Pauw, 2006; Rodriguez-Rodriguez and Valido, 2008). However, the relative effectiveness of diverse pollinators in species with generalized flowers is largely underappreciated (Larsson, 2005). Here, Asian lotus is used as an experimental model plant with generalized flowers to examine whether beetles are the most effective pollinators as predicted by its floral syndrome. If a plant with a generalized pollination system employs one type of effective pollinator although it is visited by diverse floral visitors, it provides substantial evidence to support the pollination syndrome concept. The floral biology of *N. nucifera* was investigated over a wide geographical range in China to address the following questions. (a) What are the pollinators for *N. nucifera* in different populations? (b) Which pollinators are the most effective pollinators? (c) Is the pollinator assemblage of *N. nucifera* constant across different populations?

**MATERIALS AND METHODS**

**Study species**

*Nelumbo nucifera* Gaertn (Nelumbonaceae) is an emergent aquatic plant with a wide range of distribution in the Old World throughout temperate and tropical Asia (Borsch and Barthlott, 1994). The other species in the Nelumbonaceae is the American lotus (*N. lutea* Willd.), occurring in North America. *Nelumbo nucifera*, the Asian sacred lotus, the national flower of India, is considered as one of the ten most beautiful flowers in China. It often predominates in plant communities in lakes and ponds (Wang and Zhang, 2005). The bowl-shaped flowers are hermaphroditic, solitary and large, up to 25 cm in diameter, with intense odours during anthesis. Each flower consists of two sepals and 18–28 petals, forming a chamber before the flower opens (Ni, 1987). The androecium consists of numerous stamens attached spirally to the obconical receptacle where uniovulate carpels are separately and distantly embedded (Fig. 1).

*Nelumbo nucifera* is protogynous and one flower lasts 3–4 d, undergoing changes of flower shape (Vogel and Hadacek, 2004). On the first day (hereafter termed Day-1) of flowering, the flowers are at the female stage with sticky stigmas and the petals partly open, forming a narrow tunnel above the receptacle (Seymour and Blaylock, 2000). Day-1 flowers close completely at dusk, appearing like unopened buds. The next day, the petals open again and the stamens begin to release pollen. Stigma receptivity continues during Day-2, so self-pollination may occur during the process of insect visitation. In the evening, the petals close again but much more loosely. On the third morning, the flowers open again, the stamens begin to wither and the stigmas become dry, their colour changing from yellow to brown. The petals and stamens abscise on the following days leaving the receptacles to ripen as fruit.

**Flower pollinators and visitation frequency**

Eleven wild populations across China and one cultivated population in Wuhan were investigated from 2005 to 2008 to examine diverse pollinators in *N. nucifera* (Tables 1 and 2). In each population, 10–15 Day-1 and Day-2 flowers were randomly labelled and observed in turn for 10 min censuses from 0700 to 1000 h. Insects which collected pollen and contacted stigmas were recorded as pollinators. In American lotus, beetles were observed to be commonly entrapped in closed Day-2 flowers and were considered to be important pollinators (Schneider and Buchanan, 1980). To examine whether flowers of *N. nucifera* trapped beetles or other insects to facilitate pollination, 15 closed Day-1 and Day-2 flowers were examined in the Mishan population (Heilongjiang Province, northeastern China) and the Lantian and Wuhan populations (Hubei Province, south China), respectively. Fifteen and ten Day-1 flowers were randomly collected from each of the Mishan and Wuhan populations to determine the percentage of pollinated stigmas and the number of pollen grains on each stigma under a stereomicroscope. Meanwhile, stamens, pollen grains and ovaries were counted from 15 other flowers. Unopened flowers were also bagged with small-mesh (1 × 1 mm) nets and the size (length × width) of beetles and thrips was measured to see whether these small insects could be excluded by the nets we used. In each population, at least ten fruits were randomly collected from the field to estimate the proportion of fertilized ovaries. Lotus ovaries expand after fertilization, so seed set was calculated as the ratio of expanded seeds to ovules per flower (see Table 1).

To determine the visitation frequency of pollinators for Day-1, Day-2 and Day-3 flowers, observations were made for 117 and 45 h on fine days in Mishan and Lantian populations, respectively, during 2005 and 2006. Observations were made from 0600 to 1500 h local time. In each census, the species of flower visitors, as well as their foraging behaviours, were recorded. Representative specimens of all visitors were collected and brought back for identification.

**Relative effectiveness of pollinators**

In Mishan and Lantian where beetles were absent (Table 1), bees and flies were excluded using small-mesh (1 × 1 mm) nets during 2005 and 2006 to determine the pollination role of bees and flies for *N. nucifera*. After the body size of beetles had been measured, three exclusion treatments were conducted in Wuhan in 2008 to examine the role of beetles for reproduction in *N. nucifera*: (a) excluding all pollinators with parchment paper bags (*n* = 16); (b) excluding bees, flies and some of the beetles with 1 × 1 mm small-mesh nets (*n* = 13); and (c) excluding only bees and flies, but not beetles and thrips, with 2 × 2 mm large-mesh nets (*n* = 16). Unmanipulated flowers (*n* = 16) were also collected as a control for seed production.
in open-pollinated flowers. One-way analysis of variance (ANOVA) was used to compare the value of seed set among pollination treatments and seed sets of open-pollinated flowers among 12 populations using the SPSS program. Throughout this report, all means are accompanied by their standard error unless otherwise indicated.

RESULTS

Flower visitors and visitation frequency

Four types of insects, bees, flies, thrips and beetles, were observed in Asian lotus (Tables 1 and 2, Fig. 1). Beetles were observed only in the Honghu and Wuhan populations. Bees, flies and thrips were present in all 12 populations. It was found that both thrips and beetles were trapped in closed Day-1 and Day-2 flowers. There were $10.1 \pm 1.3$ and $35.85 \pm 3.41$ (mean $\pm$ s.e.) thrips trapped in closed Day-1 ($n = 15$) and Day-2 flowers ($n = 15$) in the Mishan population, and $4.45 \pm 0.31$ and $22.82 \pm 2.4$ beetles in closed Day-1 ($n = 11$) and Day-2 flowers ($n = 11$) in the Wuhan population, respectively. The $1 \times 1$ mm small-mesh nets did not completely exclude thrips and beetles. The body length and width of thrips and beetles were $1.25 \pm 0.03 \times 0.22 \pm 0.02$ mm ($n = 11$) and $2.77 \pm 0.08 \times 1.10 \pm 0.05$ mm ($n = 15$), respectively. Thus, both thrips and beetles could easily enter the $2 \times 2$ mm large-mesh nets.
Insect visitation rates were similar in Mishan and Lantian during 2005 and 2006 (Fig. 2). The most frequent visitors were bees, including *Apis mellifera* and *Lasiosglossum* sp., accounting for 84.8% (581) of total flower visits (685) in Mishan and 97.9% (185) of total flower visits (189) in Lantian. Flower visits by flies were much less frequent compared with bees, accounting for 15.2% in Mishan and 2.1% in Lantian. Insects rarely visited Day-1 flowers before the anthers dehisced. Compared with Day-1 flowers, Day-2 flowers attracted more visitors by presenting a large amount of pollen (Fig. 2). Each bee generally spent 10–30 s in one flower collecting pollen, occasionally standing on the receptacle. Pollen adhering to bees could be deposited on stigmas when bees were flying above or on the receptacle and transferring pollen to their pollen baskets. Flies usually settled on receptacles, basking in the sunshine or feeding on the stigmatic secretion, but they rarely collected pollen. Bees or flies seldom crawled onto the receptive stigmas. The thrips, usually resting in secretion, but they rarely collected pollen. Bees or flies seldom crawled onto the receptive stigmas when bees were flying above or on the receptacle and the receptacle. Pollen adhering to bees could be deposited on stigmas with pollen deposition was very low (Table 3). The percentage of stigmas with pollen deposition was very low (Table 3). The percentage of pollinated stigmas and the mean pollen load deposited per stigma in each closed Day-1 flower were significantly lower in the Mishan population, the percentage of stigmas with pollen deposition was very low (Table 3). The percentage of pollinated stigmas and the mean pollen load deposited per stigma in each closed Day-1 flower were significantly lower in the Mishan population (where no beetles were observed, Table 1) than in Wuhan (where there were numerous beetles; *F*$_{1,24}$ = 147.98, *P* < 0.0001; *F*$_{1,35}$ = 29.99, *P* < 0.0001, respectively), indicating that beetles rather than thrips were important for cross-pollination in *N. nucifera* (Table 1).

Seed set was significantly different among treatments of flowers bagged using different mesh sizes (*F*$_{3,60}$ = 93.71, *P* < 0.0001). Flowers from which all pollinators were excluded using paper bags rarely set seeds; their mean seed set was 2.0% (Fig. 4), indicating that seed production in Asian lotus depends on pollinator visitation. The seed set of flowers caged with small-mesh nets was significantly lower than that of open-pollinated flowers, whereas the seed set of flowers bagged with large-mesh nets was not (Fig. 4). Given that small-mesh nets partly excluded beetles while large-mesh nets did not, the increase of seed set by allowing access by...
beetles suggested that beetles played an active pollination role in *N. nucifera*.

**DISCUSSION**

This study indicated that beetles were effective pollinators of *N. nucifera*, consistent with the prediction based on its floral syndrome. Furthermore, beetles were observed to be entrapped in the first-day flowers of Asian lotus, supporting the idea that this homoeothermic blossom attracts beetles with a heat reward (Seymour et al., 2003) and possibly stigmatic exudates (see Fig. 1D). We also observed other pollinators, particularly pollen-collecting bees, in accord with previous studies on the congeneric *N. lutea* (Robertson, 1889; Sohmer and Sefton, 1978; Schneider and Buchanan, 1980), and there was high seed set in some populations where no beetles were recorded, suggesting that lotus flowers could be pollinated at least by beetles and bees.

Earlier observations on Asian sacred lotus recorded only bees as pollinators in Java (Knuth, 1904) and the Caspian region (Snigirevskaja, 1964). The pollinator spectra of *N. nucifera* across China were diverse and variable (Tables 1 and 2). Beetles were found in only two populations in south China, but other insects, such as bees, flies and thrips, were usually found on *N. nucifera* from north to south in China. Furthermore, the abundance of pollinators varied among populations; for example, bees were more abundant in the Mudanjiang, Acheng, Honghu, Lantian and Wuhan populations than in the other populations (Table 1). Correspondingly, it was observed that seed sets were higher in these five populations than in populations where bees were scarce (Table 1). The assemblages of insects on *N. lutea* also varied among different sites. Robertson (1889) recorded 30 species of Hymenoptera, Diptera and Coleoptera in Illinois. Sohmer and Sefton (1978) enumerated 70 species representing ten orders and 53 families of insects in Texas. Schneider and Buchanan (1980) also listed 25 insects belonging to four orders in South central and east Texas. Although open-shaped flowers offering unrestricted access to pollen or nectar foragers are generally considered to have evolved in relation to diverse pollinators, the relative role of each pollinator remains largely unexplored. We found that *N. nucifera* could be pollinated by different bees and flies rather than exclusively by beetles, in that seed production was achieved in beetle-free populations (Tables 1 and 2), consistent with observations in *N. lutea* (Robertson, 1889; Sohmer and Sefton, 1978; Schneider and Buchanan, 1980). The flowers from which we excluded bees and flies in the (beetle-free) Mishan and Lantian populations produced lower seed sets than those of untreated flowers, suggesting that bees or flies were active pollinators. Because other insects exhibited
lower pollen loads and visitation frequencies compared with bees, we considered that bees were important pollinators of *N. nucifera* (see also Schneider and Buchanan, 1980). The observation of other insects involved in the pollination of cantharophilous *Nelumbo* is mirrored in other aquatic genera including *Nuphar* and *Nymphaea*. The three genera have been thought to be bee pollinated in that flowers are generally protogynous, bowl shaped, emitting an intense odour, producing large amounts of pollen and closing their petals in the evening. Pollinator investigations showed that bees were not the exclusive pollinators because some species or some populations of one species were pollinated by bees or flies (Ervik et al., 1995; Lippok and Renner, 1997; Lippok et al., 2000; Hirthe and Porembski, 2003). Where beetles were present, however, they did act as effective pollinators (Lippok et al., 2000; Hirthe and Porembski, 2003).

Beetle pollination has been thought to be primitive and relatively inefficient (Fægri and van der Pijl, 1979), and has been considered as a specialized system that arose in basal angiosperms (Bernhardt, 2000; Thien et al., 2009). A recent study analysing pollen carried by floral visitors to *Magnolia obovata* (Magnoliaceae) indicated that flower beetles transported large amounts of genetically diverse outcross pollen, but the pollination effectiveness of bumble-bees was low in that most pollen adhering to bumble-bees was self-pollen (Matsuki et al., 2008). We observed that visitation frequencies of bees and flies were high for Day-2 flowers (Table 3, Fig. 2). They were unlikely to visit Day-1 flowers presumably because of a lack of food rewards. The protogynous flowers on the first day of flowering have a high potential for cross-pollination compared with Day-2 flowers, in which anthers begin to dehisce. Furthermore, beetle-mediated cross-pollination could be facilitated by high stigma receptivity of Day-1 flowers (Seymour and Blaylock, 2000). In contrast to observations on Day-1 flowers of *N. lutea* where no beetles were trapped (Schneider and Buchanan, 1980), we observed beetles in closed Day-1 flowers of *N. nucifera* during the period of floral thermoregulation, confirming that this homoeothermic blossom attracts beetles with a heat reward (Seymour and Schultz-Motel, 1996, 1998; Seymour et al., 2003) or perhaps stigmatic exudates (Fig. 1D). Beetles could crawl on receptive receptacles and pollinate most of the stigmas (Table 3, Fig. 1), suggesting that beetles were effective pollinators in this chamber blossom species. The seed sets of flowers accessible to beetles were not significantly lower than that of the open-pollinated flowers (Fig. 4), indicating that beetles were important for the reproduction of *N. nucifera*, and that the role of bees and flies as pollinators could be performed by beetles. However, beetles were only observed in two of the 12 studied populations. It is not clear whether ecological disruptions eliminate beetles from these populations or if beetles are attracted to other plants given that beetles are generalist pollinators.

The flower of *N. nucifera* has been considered to be a classic example of beetle pollination (Delpino, 1875; Robertson, 1889; Bernhardt, 2000; Davis et al., 2008), with features such as large numbers of stamens without nectar, staminal appendages, actinomorphy, protogyyn, thermogenicity and movement of the perianth (opening and closing). However, the morphology of Asian lotus flowers cannot effectively exclude other pollen-collecting visitors when flowers open wide (Tables 1 and 2, Fig. 1). Although beetles were effective pollinators, supporting the pollination syndrome concept, the floral rewards are accessible to other pollinators in this generalized pollination system. One wild flower of *N. nucifera* produces around 1 million pollen grains, which is an exceptionally high number for a bee-pollinated species. Although pollen-collecting bees may be active pollinators, they are unlikely to be effective because they rarely contact the stigmas. The Asian lotus, an ancient living fossil plant, perhaps established a mutualism with beetles very early in its evolutionary history. Sexual reproduction in various populations has benefited from the other contemporary pollinators that we found.

**Table 3.** Pollen and ovule production and pollen deposition on stigmas (mean ± s.e.) in closed Day-1 flowers in two field populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Stamens per flower</th>
<th>Ovules per flower</th>
<th>Millions of pollen grains per flower</th>
<th>Pollinated stigmas per flower (%)</th>
<th>Pollen grains per pollinated stigma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mishan</td>
<td>280.25 ± 8.76</td>
<td>28.60 ± 1.13</td>
<td>1.01 ± 0.04</td>
<td>15.47 ± 3.29</td>
<td>1.30 ± 0.21</td>
</tr>
<tr>
<td>Wuhan</td>
<td>272.73 ± 6.14</td>
<td>38.15 ± 0.62</td>
<td>0.88 ± 0.02</td>
<td>84.21 ± 6.13</td>
<td>40.65 ± 9.17</td>
</tr>
</tbody>
</table>

**Fig. 4.** A comparison of seed sets (mean ± s.e.) of lotus flowers enclosed in different types of bag to examine the pollination role of beetles in the Wuhan population. The same letter indicates no significant difference among treatments.

**ACKNOWLEDGEMENTS**

The authors thank Zuo-Dong Li, En-Xing Zhou, Dong-Xu Li and Xiao-Xin Tang for their valuable help in the field, and Sarah Corbet for helpful comments on the manuscript.
A grant from the National Science Foundation of China (no. 30825005) to S.Q.H. supported this work.

LITERATURE CITED


