Breeding system and pollination of two closely related bamboo species

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Received: 25 August 2016; Editorial decision: 21 May 2017; Accepted: 27 May 2017; Published: 29 May 2017

Associate Editor: Simon J. Hiscock

Citation: Chen L-N, Cui Y-Z, Wong K-M, Li D-Z, Yang H-Q. 2017. Breeding system and pollination of two closely related bamboo species. AoB PLANTS 9: plx021; doi:10.1093/aobpla/plx021

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Abstract. An understanding of the breeding systems and pollination of agriculturally important plants is critical to germplasm improvement. Breeding system characteristics greatly influence the amount and spatial distribution of genetic variation within and amongst populations and influence the rarity and extinction vulnerability of plant species. Many woody bamboos have a long vegetative period (20–150 years) followed by gregarious monocarpy. Relatively, little is known about their pollination and breeding systems. We studied these characteristics in wild Dendrocalamus membranaceus populations and cultivated Dendrocalamus sinicus populations distributed in the Yunnan Province of China. Floral morphology, flower visitors and breeding system were studied from 2013 to 2015. Both bamboos were protogynous, but flowering periods of florets overlapped providing opportunities for self-pollination amongst florets, especially in D. membranaceus. There was no agamospermy in either species. Seed set of D. sinicus was low (0.42 ± 0.42 %) under natural pollination but higher (8.89 ± 2.55 %) after artificial xenogamy. Seed set of D. membranaceus was higher (7.49 ± 0.82 %) in mass flowering populations and 2.14 ± 0.25 % in sporadically flowering populations. The Asian honeybee Apis cerana could provide cross-pollination of D. membranaceus and D. sinicus, and flower visitation peaked at 1000–1200 h. Pollination limitation due to lack of pollinators or pollen was detected in the cultivated populations of D. sinicus and sporadically flowering populations of D. membranaceus. Pollination limitation was not obvious within mass flowering populations. Hand pollination could significantly increase seed set of these two bamboo species. Dendrocalamus membranaceus and D. sinicus were self-compatible and have a mixed-mating system with outcrossing being pre-dominant. Their seed production was limited by the quantity of pollen and pollinator activity. Honeybees were observed as effective pollinators.

Keywords: Breeding system; Dendrocalamus membranaceus; Dendrocalamus sinicus; floral morphology; pollination limitation; pollinators; woody bamboo.

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Introduction

The breeding system is a key component of plant sexual reproduction. Many studies have suggested that the health and maintenance of plant populations is strongly affected by breeding characteristics, such as flower phenology, self-compatibility and the breeding system (Nair et al. 2004; Gan et al. 2013). Understanding the reproductive biology of agriculturally important crops is crucial for developing genetic improvement strategies and establishing appropriate conservation measures (Nair et al. 2004; Rodríguez-Pérez 2005).

Bamboos (Poaceae: Bambusoideae) include ca. 88–115 genera and >1400 species. They are widely distributed, especially in the tropical and subtropical regions of the world, but absent in Europe and Antarctica (Li et al. 2006; Bamboo Phylogeny Group 2012). Bamboo species provide food and raw materials for construction, paper pulp and manufacturing. On the basis of the presence or absence of strongly lignified culms, bamboos are divided into two types: woody and herbaceous (Wysocki et al. 2015). Many woody bamboos have an unusual life cycle, characterized by a very long vegetative period of several decades to over 150 years followed by gregarious monocarp (Budke et al. 2010). In some woody bamboos, such as Ochlandra travancorica, Sasa kuriensis, Chusquea ramosissima (Venkatesh 1984; Makita 1992; Montti et al. 2011), several different types of flowering occur. These include mass flowering (the flowering of most clumps in a population over a wide range) and sporadic flowering (the flowering on a small scale or the flowering of a minority of clumps in a population). Flowering studies have mainly focused on bamboo clump dieback and the ecological impact after flowering, especially in the recruitment of associated overstory tree species following mass flowering of bamboos (Campanello et al. 2007; Baskin 2009; Giordano et al. 2009). Fewer studies have focused on the natural regeneration of woody bamboos after flowering (Xie et al. 2016). However, due to such unpredictable flowering episodes, little is known about the breeding system and pollination of woody bamboos; such knowledge would be useful for their genetic improvement, such as better adaptation to a broader range of growth conditions (Kittelson and Maron 2000; Mousset et al. 2016).

As in other grasses, wind pollination is common in bamboos (Janzen 1976; Nadgauda et al. 1993). Insect visits to bamboo flowers have been recorded frequently although the effectiveness of insects as bamboo pollinators is controversial (Janzen 1976; Venkatesh 1984; Nadgauda et al. 1993; Huang et al. 2002). However, pollination is often dependent on mutualistic interactions with visiting insects (Gan et al. 2013). Huang et al. (2002) have provided the only study documenting honeybee-assisted pollination in addition to wind pollination in bamboos. Other studies have demonstrated bamboos being dichogamous and protogynous (Venkatesh 1984; Nadgauda et al. 1993; Jijeesh et al. 2012). Unfortunately, there is no direct information as to whether bamboos are obligate outcrossers.

Dendrocalamus is a genus of woody bamboos with ~52 species (Ohrnberger 1999). The species are widely distributed in the tropical and subtropical regions of Asia (Nguyen and Xia 2013). Other than reports of flowering and floral descriptions, a detailed account of reproductive biology is lacking for any of these species. Dendrocalamus membranaceus and Dendrocalamus sinicus are large woody sympodial bamboos (Fig. 1). They are economically important as vegetable crops and for providing raw materials for furniture, construction and industrial paper pulp (Li et al. 2006). Dendrocalamus membranaceus grows in Myanmar, Laos, north Thailand, northern Vietnam and China at elevations of 500–1000 m, and is common in the Lancang-Mekong River valley (Xie et al. 2016). It is an important vegetation cover for soil and water conservation in parts of southwest China (Xie et al. 2016). Dendrocalamus sinicus is known mainly in cultivation and is endemic to Yunnan, China, in the mountainous areas of southwestern Yunnan at 500–1800 m. It has been proposed as a protected species because of its limited distribution, rarity and continuous reduction of germplasm, as well as low seed set (Yang et al. 2010; Gu et al. 2012). These two Dendrocalamus species represent a common wild species and a rare, but commonly cultivated, species (Fig. 1; Yang et al. 2010; Xie et al. 2016). Since 2008, the species have started to flower sporadically or gregariously following prolonged severe drought in Yunnan, providing an opportunity to study their reproductive biology (Xie et al. 2016).

We examined the breeding system and pollination of D. membranaceus and D. sinicus, by studying floral characters, flower visitors, and the mating systems found in mass and sporadically flowering populations. Our goals were to determine the breeding system of two woody bamboos and document aspects of the reproductive biology that may be useful for research on genetic improvement and conservation of Dendrocalamus bamboo forests.

Methods

Species characteristics and occurrence

Dendrocalamus membranaceus individuals reach heights of 10–20 m and diameters of 7–15 cm. Clumps consist of
culms sprouted from the lateral buds of shortened rhizome. Each clump contains an average of 20–30 culms and there may be >80 culms in very large clumps (An et al. 2010). In Yunnan, D. membranaceus usually develops into extensive natural bamboo forests along the lower Lancang River watershed over an area of ca. 7 × 10^4 h.m² (Xie et al. 2016). Dendrocalamus sinicus grows 20–35 m in tall and 10–30 cm in diameter and it is regarded as the strongest woody bamboo species in the world (Dong et al. 2012). Every clump contains 20–25 culms and there can be >90 culms in large clumps (Hui 2004). Local people practice small-scale cultivation of this species using clones obtained by dividing rhizomes. There was a continuous period of flowering and fruiting amongst different clumps and populations of D. membranaceus and D. sinicus in Yunnan associated with severe droughts from 2008 to 2015 (Gu et al. 2012; Xie et al. 2016). In the wild, D. membranaceus populations had both sporadic and mass flowering and fruiting, whereas only sporadic flowering and fruiting was seen in D. sinicus populations. The present study was conducted from March 2013 to December 2015 and focused on populations of D. sinicus and D. membranaceus with a similar flowering period.

### Study sites

Breeding system and pollination surveys of D. membranaceus were conducted in the bamboo-dominated forests along the Lancang River, including Xiaomengyang National Nature Reserve (XNNR) in Jinghong County (sites A and B) and Simao District of Puer City (site C; Fig. 2). The XNNR is located at 21°48′–22°20′N, 100°36′–101°22′E, with an elevation range of 600–900 m (Xie et al. 2016). The observation sites A (for mass flowering), B and C (for sporadic flowering) were selected for investigating breeding system and pollination in mass and sporadically flowering populations of D. membranaceus from the beginning of 2013. Site A was in pure D. membranaceus forest in which 52 of 61 clumps flowered (85.2 %), and four flowering clumps were randomly selected for breeding system studies. Site B was in a mixed forest of bamboo and broadleaf trees (bamboo: tree = 7:3) where 4 clumps of 32 clumps flowered (12.5 %), and all four...
flowering clumps were studied. Site C was also in a mixed bamboo-tree forest (bamboo: tree = 9:1), and 3 of 26 clumps flowered (11.5 %) (Table 1). We observed all three flowering clumps at site C. Field studies of *D. sinicus* were conducted in the cultivated populations in Menghai County of Xishuangbanna Autonomous Prefecture (site D) and Ximeng County of Puer City (site E), where *D. sinicus* was found sporadically flowering (Table 1; Fig. 2). Three and two flowering clumps were studied, respectively.

**Floret morphology and development**

We considered each bamboo clump as a probable genet and the culms within as ramets of a clone according to McClure (1966). During the full blooming stage of *D. membranaceus* and *D. sinicus*, 30 intact fresh florets were selected and measured from every clump surveyed. Floret morphological characters were observed using a ×10 hand lens. Glumes, lemmas, paleas, filaments, anthers and styles of the floret were measured for length and median width (max-width) using vernier calipers. To reveal details of floral development, such as the floral development sequence, 10 pseudospikelets from each flowering culm were observed.

**Breeding system**

To assess the effects of pollen source on seed set of *D. membranaceus* and *D. sinicus*, various pollination experiments were performed in situ during the spring of 2014 and 2015. Because the flowering bamboo clumps will die within one year, we had only one chance to apply the experimental treatments to each flowering bamboo clump. For each clump, at least five flowering culms were selected to study the breeding system. Pollination treatments, performed according to Dafni (1992) and Gan et al. (2013) were conducted as follow: natural control, no emasculation and no bagging; autonomous self-pollination, no emasculation and bagging to test whether pollinators were needed; assisted geitonogamy, emasculation and bagging to test whether geitonogamy was limited; assisted xenogamy, emasculation and bagging to test fertility of outcrossing; natural cross-pollination, emasculation and no bagging to test whether pollen transfer was limited; parthenogenesis test, emasculation, bagging and no pollination by hand to test whether agamospermy may have occurred.

Hand pollination experiments were carried out according to Zych and Stpiczyńska (2012). Pollen was collected from non-dehisced ripe anthers of either the same individual flower (self-pollination) or another flower from three unmarked clumps of the same species that was growing at a distance of at least 5 m (cross-pollination). Pollen was stored in 1.5 mL Eppendorf tubes. All pollen was applied within 4 h to ensure viability. When stigmas were visible, pollen was applied to florets using one sterile brush for each species. Three replicates of 20 pseudospikelets were marked for each treatment. For *D. membranaceus*, because only the floret at the top of a pseudospikelet was pollinated and eventually developed into a seed, we used only the topmost floret for pollination studies. In *D. sinicus*, the topmost floret of each pseudospikelet was sterile and most of pseudospikelets only bear one seed from the second floret so we only used the second floret in the hand pollination experiments. Seed set was estimated with the following formula: seed set (%) = total number of seeds/total number of pseudospikelets × 100 %.

The pollen limitation index (L; Larson and Barrett 2000) was used to quantify pollen limitation within a population for each species. The index was calculated as $L = 1 - (P_N/P_S)$, where $P_N$ is the percentage of naturally pollinated seeds and $P_S$ is the percentage seed set by supplement cross-pollen. No pollen limitation is indicated in the population if $L = 0$ (Larson and Barrett 2000).

**Flower visitors**

Flower visitor activities were observed in the field at the peak of flowering in late March of 2014 and 2015, respectively. Observations were conducted simultaneously at three sites for *D. membranaceus* and *D. sinicus* from 0800 h to 1600 h on 7 sunny days. For each observation...
we selected 10 flowering branches each having at least ten blooming pseudospikelets. Representative flower visitors were captured for identification using trap nets. It was recorded if visitors carried *D. membranaceus* and *D. sinicus* pollen. Pollinator frequency was expressed as number of visits to florets per hour.

**Statistical analysis**

Seed set of the hand pollination experiments was compared by a one-way ANOVA using PROC GLM. When significant differences were found, means were separated by Student–Newman–Keuls (SNK) multiple comparison analysis at $P = 0.01$ and $P = 0.05$, respectively. All statistical analyses were performed with SAS (Version 9.2, SAS Institute Inc., Cary, NC).

The statistical significance of pollen limitation was evaluated by Student’s *t* test (independent samples) in Excel 2010 (Microsoft Corp., Seattle, WA). Differences were considered to be statistically significant when $P \leq 0.05$ and highly significant when $P \leq 0.01$.

**Results**

**Floret morphology**

Both *D. membranaceus* and *D. sinicus* bear large-scaled panicles on leafless flower branches (Fig. 3), but they differed in floral morphology. In *D. membranaceus*, 30–70 (minimum and maximum) pseudospikelets form a dense, spiky globose mass at the nodes of flowering branches and this was 2.5–3.5 cm in diameter. Pseudospikelets are flat, ovoid, 10–12 mm long, 2–4 mm wide, yellow green when fresh and straw yellow when withered. Each pseudospikelet comprises 2–4 florets. The floret bears two ovate, apically acute glumes. Lemma is 8–9 mm long, 5–8 mm wide. Palea is membranous, 7–8 mm long, 3–6 mm wide, with two ciliate keels. Stamens protruded from a floret when mature. Filaments are milky white, 10–15 mm long. Anthers are purple, 5–8 mm long. Ovary is ovoid and hairy, and ciliate style is 10–15 mm long with one purple and plumose stigma.

In *D. sinicus*, 1–6 pseudospikelets form a sparse cluster at each node of flowering branches. Pseudospikelets are flat, narrowly ovoid, 30–45 mm long, 5–8 mm wide, purple when fresh and also straw yellow when withered. Each pseudospikelet contains 6–7 florets. Floret bears two ovate and apically acute glumes. Lemma is 10–25 mm long and 10–15 mm wide. Palea is 12–18 mm long and 6–12 mm wide, membranous, two-keeled, with a two-fid apex. Six stamens protruded from the floret when it matured. Filaments are milky white, 20–35 mm long. Anthers are 8–15 mm long, yellow, with mucronate apices. Ovary is ovoid and hairy, and ciliate style is 20–35 mm long with only one purple and plumose stigma.

**Floret development**

March 2013–December 2015 observations revealed that *D. membranaceus* and *D. sinicus* bloom at any time of the day but mostly occurred from 800 h to 1200 h. Both species were protogynous but the maturation of pistils and stamens of different florets can overlap (Fig. 4). Typically, extrusion of the stigma from the lemma occurs 4 h earlier than the stamens. In *D. sinicus*, the flowering duration of a single pseudospikelet (from protrusion of the stigma of the uppermost fertile floret to withering of

![Figure 3. Inflorescences and hand pollination experiments of *D. membranaceus* (A–D) and *D. sinicus* (E–H).](image-url)
the stigma of the last maturing floret) lasts up to 30 h, which was 10 h longer than that of *D. membranaceus*.

Florets in the same pseudospikelet matured at different times. In *D. membranaceus*, the topmost floret matured first, but the basal florets often degenerated. In *D. sinicus*, the topmost floret was vestigial, and florets matured sequentially from the second floret downwards, i.e. in a basipetal manner (Fig. 4).

**Breeding system**

Seeds were produced after the geitonogamy and xenogamy fertilization treatments (Fig. 5), indicating that *D. membranaceus* and *D. sinicus* are self-compatible and outcrossing fertile. However, results from a one-way ANOVA indicated that a highly significant difference of seed set ($F_{17, 36} = 60.43, P < 0.0001$) existed between the assisted geitonogamy and assisted xenogamy in five study sites (sites A–E). These sites included mass flowering populations and sporadically flowering populations of *D. membranaceus* as well as sporadically flowering populations of *D. sinicus*. This suggests that these two species are pre-dominantly outcrossing ($P < 0.01$). Seed set from assisted geitonogamy was significantly higher ($P < 0.05$) than seed set from autonomous self-pollination, and there was no significant difference between natural pollination and autonomous self-pollination and natural cross-pollination (Fig. 5), suggesting pollination limitation in those populations. No seeds were obtained with emasculation and bagging, suggesting absence of agamospermy in two bamboo species.

Seed set in *D. sinicus* was $0.42 \pm 0.42\%$ under natural pollination in sites D and E. After artificial xenogamy, seed set increased 20-fold to $8.89 \pm 2.55\%$. Seed set of *D. membranaceus* was relatively higher. Results from a second ANOVA revealed that there was a significant difference between mass flowering ($7.49 \pm 0.82\%$) and sporadically flowering ($2.14 \pm 0.25\%$) populations with natural pollination ($F_{11, 24} = 63.86, P < 0.0001$; Fig. 5). There was also a significant difference in seed set from assisted xenogamy and natural cross-pollination ($P < 0.01$) between flowering populations of *D. membranaceus* ($F_{11, 24} = 63.86, P < 0.0001$; Fig. 5), but no significant differences in seed set from autonomous self-pollination and assisted geitonogamy.

**Pollination limitation**

Pollen limitation (based on comparison of natural pollination with supplemental pollination) occurred in sporadically flowering natural populations of *D. membranaceus* as well as in the cultivated stand of *D. sinicus*. The pollen limitation index ($L$) values were 0.895 and 0.961,
respectively (Table 2), and the difference was highly significant ($P < 0.01$) in *D. sinicus*, which had higher mean seed set with supplemental pollination ($10.67 \pm 0.82$) compared with open pollination ($0.42 \pm 0.42$). In the mass flowering *D. membranaceus* population there was no significant pollen limitation although seed set under natural pollination ($7.49 \pm 0.82$) was significantly increased seed set ($31.17 \pm 0.63$) in the supplemented treatment.

### Flower visitors

Although both *D. membranaceus* and *D. sinicus* were expected to be wind pollinated, insect flower visitors were observed at five study sites. We observed a total of 48 and 82 insect visits to *D. membranaceus* and *D. sinicus* flowers, respectively. The Asian honeybee *Apis cerana* was the most common insect visitor (Fig. 6). On sunny days, the mean visitation frequency of *A. cerana* was 11.5 and 19.5 times per hour for *D. membranaceus* and *D. sinicus*, respectively. Visitation frequency peaked from 1000 h to 1200 h for both bamboo species. Thereafter, the number of visiting bees rapidly declined. Cloudy or rainy days also reduced visitation frequency (Fig. 6).

Honeybees were often observed visiting more than one flower per clump, and generally had short flight ranges. Most visitors grasped anthers and gathered pollen grains with their forelegs, then transferred grains to...
their hindlegs and abdomen. At the same time, masses of grains were dispersed into the air, accentuated when more bees visited flowers.

Discussion
Breeding system
Dendrocalamus membranaceus and D. sinicus are self-compatible but pre-dominantly outcrossing, and no apomixis occurs. The results are consistent with the findings of Venkatesh (1984) and Kitamura and Takayuki (2011) who reported that bamboo species such as Bambusa arundinacea and Sasa cernua, were self-compatible because seed set was observed even with imposed (bagged) isolation and without emasculation. In this study, the seed set from xenogamy and the control was greater than those from autogamy, suggesting that outcrossing is pre-dominant in the breeding system of D. membranaceus and D. sinicus and similar to other bamboos such as those reported by Gan et al. (2013). Higher seed set in the two species from assisted geitonogamy compared with autonomous self-pollination implied that self-fertilization may depend on pollen vectors. The seed set from autonomous self-pollination and assisted geitonogamy were similar in mass and sporadic flowering populations of D. membranaceus, suggesting stable self-fertility. But in D. sinicus, the self-compatibility may be dependent on pollen vectors such as honeybees because bagging experiments confirmed the absence of autofertility. These results are similar with those of Johnson et al. (2006) and Blambert et al. (2016).

Mating systems influence the ecology and evolutionary dynamics of plant populations (Kittelson and Maron 2000; Nair et al. 2004; Mousset et al. 2016). Many plant species employ a mixed mating strategy (Lord 2015), and this has been reported for the Poaceae such as S. cernua (Kitamura and Takayuki 2011) and Sorghum bicolor (Muraya et al. 2011). Dendrocalamus membranaceus and D. sinicus also demonstrate a mixed mating system with pre-dominant outcrossing. They are both self-compatible but pollinator-dependent in D. sinicus and less so in D. membranaceus.

Pollination
Pollination of seed plants is an essential step in their sexual reproduction (Memmott et al. 2007). Dendrocalamus membranaceus and D. sinicus are wind pollinated, as in most woody bamboos such as Phyllostachys pubescens (Cheung et al. 1985), Dendrocalamus strictus (Nadgauda et al. 1993) and Arundinaria gigantea (Gagnon and Platt 2008). However, pollinators such as Apis mellifera, have been observed visiting the flowers of some bamboos (Venkatesh 1984; Nadgauda et al. 1993). Their role in the pollination of bamboos was overlooked because they only seemed to gather pollen in the male phase of flowering and neglected the female phase. As such, they were only considered to be vectors of pollen (Nadgauda et al. 1993). In our study, honeybees at a site visited flowers sequentially and carried pollen away on their hindlegs, increasing the likelihood of transferring pollen from flowers of the same or nearby flowering bamboo clumps. Honeybees may be good pollinators for D. membranaceus and D. sinicus. These two bamboo species may have evolved to release pollen that is available for bee pollination. The time of pollen release may coincide with the peak of their flower visitation activity. Masses of pollen grains were released into the air when bees visited flowers and this would increase the chances for geitonogamy pollination. Our observations suggest that honeybee pollination could augment wind-pollination in D. membranaceus and D. sinicus, similar to study on Phyllostachys nidularia by Huang et al. (2002).

Natural vs. artificial population variation in breeding system
Habitat fragmentation and destruction affect plant reproduction by reducing the availability, and increasing the distance between, potential mates (Larson and Barrett 2000; Blambert et al. 2016). In the present study, the seed set of D. sinicus was significantly lower than that of the mass-flowering population of D. membranaceus, but was similar to that of the sporadically flowering populations of the latter. Pollen limitation may have
contributed to the low seed set but this could be overcome by assisted pollination, and the increase was more evident in *D. sinicus*. Generally, pollen limitation results from a reduction in the quantity and/or quality of pollen deposited on stigmas, leading to lower ovule fertilization and seed production (*Gonzalez-Varo et al. 2009; Aguiló et al. 2015*). In the present study, the spatial distribution of individuals in a population and pollinator presence and activity, may have helped to reduce pollen limitation. These factors also influenced pollination in *Phylloclade aleutica* (*Kameyama and Kudo 2009*). The spatial separation of flowering clumps could reduce opportunities for outcrossing in both the sporadic flowering cultivated stands of *D. sinicus* and the natural populations of *D. membranaceus*. Inbreeding, through self-fertilization, may cause an increase in homozygosity amongst offspring, leading to accumulation of deleterious alleles and inbreeding depression (*Blambert et al. 2016; Xie et al. 2016*). Such inbreeding depression can be expressed as postzygotically by reduced seed germination in bamboo (*Kitamura and Takayuki 2011*). Yang et al. (2013) found that seeds of *D. membranaceus* had a higher germination rate (81.0 %) than those of *D. sinicus* (43.3 %), though it was unclear if inbreeding depression was involved.

Conservation biology

Given the rapid decline of *D. membranaceus* and *D. sinicus* populations (*Gu et al. 2012; Xie et al. 2016*), immediate conservation efforts should be considered to protect their germplasm. The genetic diversity of the species would be at risk of further decline, so both in situ and ex situ conservation measures may be necessary for these species. For *D. membranaceus*, management of the species could involve enlarging or retaining populations with genetically less-related individuals, i.e. ‘genetic rescue’ (*Holmes et al. 2008*). Meanwhile, closing the land for reforestation and prohibiting human intrusion would help natural recovery processes in the mass-flowering populations (*Xie et al. 2016*). Attention should be directed toward the conservation of *D. sinicus* due to its limited distribution and unsupervised collection of culms (*Hui 2004*). One practical conservation solution would be to establish more ex situ cultivated stands to meet commercial demands. We should also make a special effort to collect seeds of *D. membranaceus* and *D. sinicus* for study. More research is needed to determine whether inbreeding depression is occurring in their flowering populations, especially in the sporadic flowering populations.

Conclusions

This is the first report on variation in breeding system from mass and sporadically flowering bamboo populations. Our results demonstrate that both *Dendrocalamus* species have a mixed mating system with self-compatibility, predominant outcrossing and no agamospermy. The selfing component may be dependent on a pollen vector for seed setting, particularly in *D. sinicus*. Reproductive limitations were revealed in the sporadically flowering populations of *D. sinicus*. Pollen limitation and scarcity of wild pollinators influenced the sexual reproduction of *D. membranaceus* and *D. sinicus*. Honeybees may effectively augment wind pollination in sporadically flowering populations of both species.

Sources of Funding

The research was supported by the Fundamental Research Funds of the Chinese Academy of Forestry (CAFYBB2017ZX001-8), the National Natural Science Foundation of China (31270662), Department of Sciences and Technology of Yunnan Province (2014HB041, 2008OC001).

Contribution by the Authors

L.-N.C., Y.-Z.C. and H.-Q.Y. conducted experiment work, analysed the data; L.-N.C., Y.-Z.C., K.-M.W. and H.-Q.Y. wrote the paper; H.-Q.Y. and D.-Z.L. conceived the experiments.

Conflicts of Interest Statement

None declared.

Acknowledgements

We thank Dr Simon J. Hiscock (University of Oxford, UK), Dr J. Hall Cushman (Sonoma State University) and two anonymous reviewers for their valuable comments and suggestions.

Literature Cited

Aguiló JC, Pérez-Boñón C, Crespo MB, Juan A. 2015. Puzzling out the reproductive biology of the endangered cat’s head rockrose (*Helianthemum caput-felis*, Cistaceae). *Flora* 217:75–81.

An MY, Yang HQ, Yang YM, Sun MS. 2010. Preliminary study on resources and protection of natural *Dendrocalamus membranaceus* forest in China. *Shandong Forestry Science and Technology* 4:111–114.

Bamboo Phylogeny Group. 2012. An updated tribal and subtribal classification for the Bambusoideae (Poaceae). In: Giels J,
Potters G, eds. Proceedings of the 9th world bamboo congress. Antwerp, Belgium: World Bamboo Organization.

Barrett SC. 1998. The evolution of mating strategies in flowering plants. Trends in Plant Science 3:335–341.

Baskin JM. 2009. Death of Bamboo triggers regeneration of overstory tree in a southern beech forest. New Phytologist 181:749–750.

Blambert L, Mallet B, Humeau L, Poiller T. 2016. Reproductive patterns, genetic diversity and inbreeding depression in two closely related Jumellea species with contrasting patterns of commonness and distribution. Annals of Botany 118:93–103.

Budke JC, Alberti MS, Zanardi C, Baratto C, Zanin EM. 2010. Bamboo dieback and tree regeneration responses in a subtropical forest of South America. Forest Ecology and Management 260:1345–1349.

Campanello PI, Gatti MG, Ares A, Montti L, Goldstein G. 2007. Tree regeneration and microclimate in a liana and bamboo-dominated semideciduous Atlantic forest. Forest Ecology and Management 252:108–117.

Cheung YY, Copper SG, Hansken TJ, Cheung YC. 1985. In: Research on the raising of Phyllostachys pubescens seedling. In: Recent research on bamboo. Proceedings of the International Workshop Recent Research on Bamboos. Hangzhou, China. IDRC, Ottawa.

Dafi A. 1992. Pollination ecology: a practical approach. Oxford, England: IRL Press Ltd.

Dong YR, Zhang ZR, Yang HQ. 2012. Sixteen novel microsatellite loci from Dendrocalamus sinicus (Poaceae). The strongest woody bamboo in the world. American Journal of Botany 99:e347–e349.

Gagnon PR, Platt WJ. 2008. Reproductive and seedling ecology of a semelparous native bamboo (Arundinaria gigantea, Poaceae). The Journal of the Torrey Botanical Society 135:309–316.

Gan X, Cao L, Zhang X, Li H. 2013. Floral biology, breeding system and pollination ecology of an endangered tree Tetracentron kurilensis Oliv. (Trochodendraceae). Botanical Studies 54:50.

Giordano CV, Sánchez RA, Austin AT. 2009. Gregarious bamboo flowering opens a window of opportunity for regeneration in a temperate forest of Patagonia. New Phytologist 181:880–889.

González-Varo JP, Arroyo J, Aparicio A. 2009. Effects of fragmentation on pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (Myrtus communis). Biological Conservation 142:1058–1065.

Gu ZJ, Yang HQ, Sun MS, Yang YM. 2012. Distribution characteristics, flowering and seeding of Dendrocalamus sinicus in Yunnan, China. Forest Research 25:1–5.

Holmes GD, James EA, Hoffman AA. 2008. Limitations to reproductive output and genetic rescue in populations of the rare shrub Grevillea repens (Proteaceae). Annals of Botany 102:1031–1041.

Huang SQ, Yang HF, Lu J, Tokahashi Y. 2002. Honeybee-assisted wind pollination in bamboo Phyllostachys nidulata (Bambusoideae: Poaceae)? Botanical Journal of the Linnean Society 138:1–7.

Hui. 2004. A study on the population characteristics of the valuable and rare bamboo species of Dendrocalamus sinicus and its conservation in Yunnan, China. Journal of Bamboo Research 23:4–8.

Jacquemyn H, Micheneau C, Roberts DL, Poiller T. 2005. Elevational gradients of species diversity, breeding system and floral traits of orchid species on Reunion Island. Journal of Biogeography 32:1751–1761.

Janzen DH. 1976. Why bamboos wait so long to flower. Annual Review of Ecology and Systematics 7:347–391.

Jiweesh GM, Seethalakshmi KK, Raveendran VP. 2012. Flowering, reproductive biology and post flowering behaviour of Dendrocalamus sikkimensis Gamble, in Kerala, India. Bamboo Science and Culture 25:36–42.

Johnson SD, Hargreaves AL, Brown M. 2006. Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. Ecology 87:2709–2716.

Kameyama Y, Kudo G. 2009. Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, Phyllodoce aleutica: effects of pollinators and self-incompatibility. Annals of Botany 103:1385–1394.

Kitamura K, Takayuki K. 2011. Estimation of outcrossing rates at small-scale flowering sites of the dwarf bamboo species, Sasa cernua. Journal of Plant Research 124:683–688.

Kittelson PM, Maron JL. 2000. Outcrossing rate and inbreeding depression in the perennial yellow bush lupine, Lupinus arboreus (Fabaceae). American Journal of Botany 87:652–660.

Larson BM, Barrett SC. 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69:503–520.

Li DZ, Wang ZP, Zhu ZD, Xia NH, Jia LZ, Guo ZH, Yang GY, Stapleton C. 2006. Bamboosea. In: Wu ZY, Raven PH, Hong DY, eds. Flora of China. Beijing: Science Press; Missouri Botanic Garden Press, 7–180.

Lord JM. 2015. Patterns in floral traits and plant breeding systems on Southern Ocean Islands. AoB PLANTS 7:plv095.

Makita A. 1992. Survivorship of a monocarpic bamboo grass, Sasa kurilensis, during the early regeneration process after mass flowering. Ecological Research 7:245–254.

McClure FA. 1966. The bamboo, a fresh perspective. Cambridge, MA: Harvard University Press, 1–362.

Memmott J, Craze PG, Waser NM, Price MV. 2007. Global warming and the disruption of plant-pollinator interactions. Ecology Letters 10:710–717.

Montti L, Campanello PI, Goldstein G. 2011. Flowering, die-back and recovery of a semelparous woody bamboo in the Atlantic Forest. Acta Oecologica 37:361–368.

Mousset M, David P, Petit C, Pouzadoux J, Hatt C, Flaven E, Ronze O, Mignot A. 2016. Lower selfing rates in metallicolous populations than in non-metallicolous populations of the pseudometallophyte Noccaea coerulescens (Brassicaceae) in Southern France. Annals of Botany 117:507–519.

Muraya MM, Mutegi E, Geiger HH, de Villiers SM, Sagnard F, Kanyenji BM, Kiambi D, Parzies HK. 2011. Wild sorghum from different eco-geographic regions of Kenya display a mixed mating system. Theoretical and Applied Genetics 122:1631–1639.

Nadgavda RS, John CK, Mascarinho AS. 1993. Floral biology and breeding behavior in the bamboo Dendrocalamus strictus Nees. Tree Physiology 13:401–408.

Nair RM, Dundas IS, Wallwork M, Verlin DC, Waterhouse L, Dowling E, Ronce O, Mignot A. 2016. Lower selfing rates in metallicolous populations than in non-metallicolous populations of the pseudometallophyte Noccaea coerulescens (Brassicaceae) in Southern France. Annals of Botany 117:507–519.

Nair RM, Dundas IS, Wallwork M, Verlin DC, Waterhouse L, Dowling E, Ronce O, Mignot A. 2016. Lower selfing rates in metallicolous populations than in non-metallicolous populations of the pseudometallophyte Noccaea coerulescens (Brassicaceae) in Southern France. Annals of Botany 117:507–519.
Ohmberger D. 1999. The bamboos of the world: Annotated nomenclature and literature of the species and higher and lower taxa. Elsevier Science BV, Amsterdam, New York, Oxford, Tokyo.

Rodríguez-Pérez J. 2005. Breeding system, flower visitors and seedling survival of two endangered species of Helianthemum (Cistaceae). Annals of Botany 95:1229–1236.

Venkatesh CS. 1984. Dichogamy and breeding system in a tropical bamboo Ochlandra travancorica. Biotropica 16:309–312.

Wysocki WP, Clark LG, Attigala L, Ruiz-Sanchez E, Duvall MR. 2015. Evolution of the bamboos (Bambusoideae; Poaceae): a full plastome phylogenomic analysis. BMC Evolutionary Biology 15:1.

Xie N, Chen LN, Wong KM, Cui YZ, Yang HQ. 2016. Seed set and natural regeneration of Dendrocalamus membranaceus Munro after mass and sporadic flowering in Yunnan, China. PLoS ONE 11:e0153845.

Yang HQ, Liang N, Li CF, Li TC, Sun MS. 2013. Seed germination and storage of six woody bamboo species in Yunnan, China. Forest Research 26:710–714.

Yang HQ, Ruan ZY, Tian B, Yang YM, Sun MS. 2010. Genetic differentiation of four straight-culmed provenances of Dendrocalamus sinicus based on ISSRs. Journal of Zhejiang Forestry College 27:81–86.

Zych M, Stpiczyńska M. 2012. Neither protogynous nor obligatory out-crossed: pollination biology and breeding system of the European Red List Fritillaria meleagris L. (Liliaceae). Plant Biology 14:285–294.