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ORIGINAL PAPER

In-situ and ex situ pollination biology of the four threatened plant species and the significance for conservation

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Abstract

Both in situ and ex situ conservation are important strategies for protecting threatened plant species. Nevertheless, the success of conservation depends on whether the plant species can naturally regenerate and accomplish its life cycle over a long-term. Here we studied the pollination biology of the threatened species *Hibiscus aridicola*, *Amorphophallus albus*, *Stemona parviflora* and *S. japonica* aiming to get data about pollination strategies, pollinators as well as mating systems. These experiments were performed at Kunming Botanical Garden (KBG) for ex situ and the plant species' natural habitat for in situ conservation. The results indicated that *H. aridicola* is self-compatible and had pollinators under both ex situ and in situ conditions. The other three species are all self-incompatible and a limited number of pollinators for *S. parviflora* and *S. japonica* were observed at cultivated and natural habitats. *Amorphophallus albus* had no pollinators at KBG but a large number of rove beetles (*Atheta* sp.) could be observed in the plant species' natural habitat. This resulted in a high fruit set under natural conditions (73.3%, $n = 30$). The results showed clearly, that appropriate pollinators for the four plant species are not present all the time and all localities, which further influences the reproduction success of a plant species. Hence, for a successful conservation, it is vital to assess the species reproduction strategy prior deciding whether in situ and/or ex situ conservation should be carried out.

Keywords *Amorphophallus albus* · Ex situ and in situ conservation biology · *Hibiscus aridicola* · Mating system · Pollination · *Stemona japonica* · *Stemona parviflora*

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Introduction

Plants as autotrophic organisms are essential to life on earth including human beings for providing fundamental materials like food, medicines, fuels etc. Apart that, they also absorb carbon dioxide, purify water and air to sustain our ecosystem (Sanders 2007; Buckley 2011; Mounce et al. 2017). However, biodiversity is being reduced severely due to human activities including urbanization, environmental pollution etc. (Jackson and Kennedy 2009; Dirzo et al. 2014; Vila et al. 2011; Socolar et al. 2016). According to the recently updated IUCN red list (https://www.iucnredlist.org/resources/summary-statistics) there are 13,494 threatened species (47.7%) among 28,287 assessed species in major taxonomic plant groups. It was evidenced that loss of biodiversity impacts primary production and decomposition processes directly. These are indispensable processes of the carbon cycle and supply many ecosystem services (Hooper et al. 2012; Reich et al. 2012).

At present, many methods are applied for protecting threatened plant species because conservation biology is getting more and more concerned (Pukazhenthi et al. 2006; Cha-Um et al. 2007; Engelmann 2011; Gundu and Adia 2014; Gashi et al. 2015). Among them, in situ conservation in natural reserves and ex situ conservation in botanical gardens or arboreta are the most important and direct ways (Green 2006; Huang 2011). In China, more than 3000 nature reserves covering approximately 16% of the land area have been established in past decades (Huang 2011). Botanical gardens also play a vital role in ex situ conservation (Odonnell and Sharrock 2017). To date, at least 105,643 species and over 40% of known threatened species are conserved in about 2500 botanical gardens worldwide (Golding et al. 2010; Mounce et al. 2017).

Although botanical gardens and natural reserves have collected and conserved many threatened plant species, but, whether these species receive effective conservation conditions depends on long-term monitoring and research (Kang et al. 2011; Canessa et al. 2016). Li et al. (2005) compared the difference of genetic diversity about *Parashorea chinensis* (Dipterocarpaceae) under in situ and ex situ conservation and found that the populations protected in nature reserve areas possessed most of the genetic variation of the species while ex situ conserved population did not contain enough genetic variation to meet the need of release this species in near future. Pollination and seed dispersal of threatened plants cultivated in botanical gardens are often overlooked despite they play an important role in plant reproduction (Calviño-Cancela et al. 2012; Neuschulz et al. 2016). Moreover, the success of pollination and seed dispersal of threatened species is closely related to the abundance of proper pollinators and seed dispersers (Ma et al. 2013; Caughlin et al. 2015). Several preliminary studies about pollination of threatened species in botanical gardens showed that those species are either self-compatible or their pollinators can survive well (Chen et al. 2011; Zhang and Ye 2011; Krestova and Nesterova 2013; Chen et al. 2015, 2019). On the contrary, lack of pollinators and/or seed dispersers of threatened species may lead to a decline of species fitness, diversity and even to extinction (Biesmeijer et al. 2006). In botanical gardens, many threatened species cannot propagate successfully due to the lack of pollinators and/or seed dispersers (Gong et al. 1998; Gong et al. 2003). Hence, it is necessary for researchers in botanical gardens to concern and study the pollination and seed dispersal strategies of threatened plant species.

For the present work, we chose the four threatened species *Hibiscus aridicola* (J. Anthony), Malvaceae, *Amorphophallus albus* (P.Y. Liu & J.F. Chen), Araceae, *Stemona parviflora* (C.H. Wright) and *S. japonica* ((Blume) Miq.), both Stemonaceae, all of them endemic to China, to study their pollination biology under in situ and ex situ conditions.

Hibiscus aridicola and *A. albus*, both are endemic to dry and hot valleys along the Jinsha River, were listed as the targeted PSESP (Plant Species with Extremely Small Populations) for the China national key program of Survey and Germplasm Conservation of Plant Species with Extremely Small Populations in Southwest China (Yang and Sun 2017). *Hibiscus aridicola*, a deciduous shrub, usually grows in steep cliffs along the Jinsha River and was destroyed badly by humans activities due to its highly ornamental value in the last years. Currently, its status of endangered has been evaluated as vulnerable [VU A2c; B2ab(i,ii,iii)] by Threatened Species List of China's Higher Plants following IUCN Red List Categories and Criteria (Qin et al. 2017). *Amorphophallus albus* is a perennial herb and grows in the open forests or arid thickets above 800 m sea level in the northeast of Yunnan and south of Sichuan and is used as a crop plant due to its edible tubers (Li and Hetterscheid 2010). The two other studied species are vines and their roots are excavated by humans due to the importance in the traditional medicine (Chen et al. 2019). *Stemona parviflora* is endemically to Hainan Province whilst *S. japonica* is occurring in the east of China (Ji and Duyfjes 2000). In this study, we explored the pollination biology and mating systems of each species in order to reveal whether ex situ conservation at KBG is suitable for these species. For comparison, we assessed also the pollination success under in situ conservation conditions in their native habitats. The results are discussed briefly in context of plant conservation.

Materials and methods

Locations

The investigation of the mating system and pollination biology of the four species were conducted during 2011–2018. For ex situ pollination experiments, all of the four species were cultivated at KBG (25° 8′ 49″ N and 102° 44′ 41″ E, 1788 m). Each of the plant species grew well and flowered every year normally. For pollination experiments in the natural habitats, *H. aridicola* (27° 14′ 45″ N and 102° 52′ 59″ E, 835 m), ca. 50 individuals with > 2500 flowers per year and *A. albus* (27° 38′ 21″ N and 103° 13′ 53″ E, 1404 m), ca. 200 individuals (> 100 inflorescences), both located at Jinyang Country, Sichuan Province, China, were chosen. The native population of *S. parviflora* for these experiments was located at Nankai village, Hainan Province, China (19° 01′ 46″ N and 109° 23′ 39″ E, 300 m) with more than 80 individuals and more than 500 flowers. The two natural populations of *S. japonica* were located at Dingjiashan, on a hill adjacent to Hangzhou City (30° 17′ 35″ N and 120° 8′ 9″ E, 53 m) and the Tianmushan National Natural Reserve (30° 19′ 15″ N and 119° 26′ 2″ E, 437 m), Zhejiang Province, Eastern China.

Pollinator observations

All the pollinator observations of the four species at KBG and in their natural habitats were performed during 2011–2018. Among the four species, pollinator observations on *H. aridicola* in the wild were conducted from 8 a.m. to 6 p.m. during its flowering period from July to October in 2011–2012. For each observation, we chose 4–6 plants randomly and continued for 30 min every hour. Overall, the accumulated observation time was more than 80 h. Observations on *H. aridicola* at KBG were conducted the same daytime as in the native habitat between July and October in 2017–2018. Each of the observers chose randomly an area including 6–8 flowers. The total observation time was more than 120 h in this location. For *A. albus*, it starts blooming about 6 a.m. so pollinator observations, both at KBG and in the native habitat, were carried out from 6 a.m. to 5 p.m. during June to July in 2016–2018. Each observation lasted 30 min every 2 h over more than 10 days. Additionally, pollinator observations of *S. parviflora* in the wild were done from 8 a.m. to 5 p.m. during its flowering period from April to March in 2010–2011. In total, an accumulation time of more than 48 h was achieved. The floral visitors for *S. japonica* in their natural habitat were observed continuously during the flower opening time from 9:30 to 18:30 for 6 days between July 9th and 14th. These observations were conducted in parallel by three observers (3 × 5 flowers observed per day). Besides that, pollinator observations on *S. parviflora* and *S. japonica* at KBG were conducted together from 9:00 to 17:00 in July to August for more than 20 days. During flower/inflorescence visitor observations of the four species, the behavior of flower/inflorescence visitors including movements on the flowers/inflorescences or any contact with floral organs were recorded. Additionally, we recorded whether the visitors carried pollen. This was done by using the optical microscope (OLYMPUS-BX51). For identification of the insects, we caught pollinators and preserved or photographed them for later identification by insect taxonomists.

Mating systems

Four experiments to test mating systems were designed for the four species, therefore flowers/inflorescences were selected randomly from each population. The treatments were as follows: (1) selected flowers/inflorescences were untreated and served as natural control; (2) selected flowers/inflorescences were tagged and closed in nylon nets (one flower/inflorescence per net) to exclude visitors and allow only self-pollination; (3) bagging selected flowers/inflorescences before anthesis and doing hand-pollinated by their own pollen after opening, then bagged again; (4) bagging selected flowers/inflorescences before anthesis and performing hand-pollinated by collected pollen from other individuals growing in a distance of more than 20 m, emasculated and bagging again. All the treatments were conducted during their full-blooming stage and on sunny days.

Data analysis

All the collected data of the fruit sets of the four treatments under different locations or time were tested by Chi square test using SAS 9.4.

Results

Pollinators

Hibiscus aridicola

During our observations, we found that no effective pollinators appeared at KBG before August, but some visitors like *Apis cerana*, *Apis mellifera ligustica*, *Byasa confusa* and some beetles showed up occasionally. During August, *Xylocopa appendiculata* appeared regularly and became the major pollinator of this plant species (Fig. 1a). This carpenter

Fig. 1 Pollinators of four threatened plant species in this study: **a** *Xylocopa* sp. pollinator of *H. aridicola*; **b** *Atherigona* sp. pollinator of *S. japonica*; **c** fly pollinator of *S. parviflora*; **d** *Atheta* sp. pollinators of *A. albus*

bee species is relatively large in size with a fast speed and strong pollen carrying capacity. Their visiting time per flower per hour was 0.98 ± 0.70 s with an average visiting time of 2.68 ± 2.16 s. The major pollinator in the native habitat was also *X. appendiculata* with a visiting time of about 0.75 ± 0.51 s per flower per hour mainly between noon and 2 p.m. Other flower visitors observed were *Cletus punctiger* and *Papilio xuthus*, carrying a few pollen grains occasionally on their bodies.

Amorphophallus albus

The results of our observations indicated that this plant species might have no pollinators at KBG. Only some flies like *Sarcophaga* sp., *Calliphora* sp., *Musca* sp. landed on the spathe occasionally and did not enter the inflorescence. In the native habitat a lot of rove beetles (*Atheta* sp.) could be observed. They gathered together in the inflorescences at its female phase (Fig. 1d). An average number of rove beetles of about 563 ± 293 ($n = 25$) per inflorescence could be assessed. These rove beetles were feeding on the sterile area of the inflorescence or mating reciprocally until the dehiscence of the anthers started. After that they moved to another inflorescence in female stage carrying pollen grains. Thus, cross pollination of *A. albus* was carried out perfectly by these rove beetles.

Stemona parviflora and *S. japonica*

During our observations, we found that no pollinators showed up for both plant species at KBG, only *Aphiochaeta* sp. visited them (Fig. 1b) with a visiting time of *S. parviflora* about 0.06 s per flower per hour and 0.13 s for *S. japonica*. Other visitors were flies like *Drosophila* sp., *Labidura riparia* and Anthomyiidae, but they just remained on the petals and did not touch the stamens. From latter species, 114 insects belonging to different families were counted in the plants' native habitat. Most of them were dipterans belonged to the five families Muscidae (98), Sarcophagidae (6), Anthomyiidae (4), Lauxaniidae (3) and Tachinidae (3), whereas 94 individuals (82.4%) belonged to the two *Atherigona* species (genus Muscidae). Their visiting time per flower per hour was 0.09 s in 2011 and 0.06 s in 2012 (Chen et al. 2015). For *S. parviflora*, no occasional flower visitors were observed during these studies. Pollination was mainly done by small flies (Fig. 1c).

Table 1 Fruit sets and seed sets of *H. aridicola*

Mating system test	KBG		Wild	
	June (no *Xylocopa*) (%)	August (*Xylocopa*) (%)	Fruit set (%)	Seed set (%)
Natural pollination	5.9	44.0	30.2 ± 4.2	16.8 ± 6.0
Bagging	2.0	8.0	11.1 ± 4.2	17.5 ± 1.9
Artificial self-pollination	34.0	48.0	67.4 ± 2.4	17.4 ± 3.4
Artificial cross-pollination	77.2	78.0	95.0 ± 1.0	27.8 ± 4.5

Table 2 Fruit sets and seed sets of *A. albus*

Mating system test	KBG		Wild	
	Fruit set (%)	Seed set (%)	Fruit set (%)	Seed set (%)
Natural pollination	0	–	73.3	47.0 ± 11.5
Bagging	0	–	0	–
Artificial self-pollination	0	–	0	–
Artificial cross-pollination	83.3	43.9 ± 9.6	80.0	64.1 ± 4.0

Mating systems

Hibiscus aridicola

The fruit sets of all the treatments in June were lower than in August at KBG. In June, the highest fruit set was reached by artificial cross-pollination (77.2%, $n=57$) followed by artificial self-pollination (34.0%, $n=50$) while the fruit set of natural pollination (5.9%, $n=51$) and bagging (2.0%, $n=51$) were rather low. In August, the highest fruit set was achieved by artificial cross-pollination (78.0%, $n=50$) followed by artificial self-pollination (48.0%, $n=50$) and natural pollination (44.0%, $n=50$). Bagging showed the lowest fruit set (8.0%, $n=50$). Among the treatments in the wild, artificial self-pollination led to the maximum fruit set of 95.0 ± 1.0% ($n=75$) with a seed set of 27.8 ± 4.5%. Artificial self-pollination and natural pollination led to fruit sets of 67.4 ± 2.4% ($n=75$) and 30.2 ± 4.2% ($n=75$), respectively. When compared fruit sets of the four treatments at KBG to the wild, natural pollination ($\chi^2=0.46$, $P>0.05$) and bagging at KBG ($\chi^2=2.83$, $P>0.05$) showed no significant differences to the wild, whilst artificial self-pollination ($\chi^2=11.31$, $P<0.001$) and artificial cross-pollination ($\chi^2=7.89$, $P<0.05$) at KBG differed significantly to the wild. The detailed data and statistics test results of *H. aridicola* are given in Tables 1 and 4.

Amorphophallus albus

The fruit sets of *A. albus* of bagging and artificial self-pollination both at KBG and in the native habitat were 0 ($n=30$) (Table 2). Also, the fruit set of natural pollination at KBG was 0 ($n=30$) in contrast to the natural habitat with 73.3% ($n=30$) and a seed set of

Table 3 Fruit sets of *S. parviflora and S. japonica*

Mating system test	*S. parviflora*			*S. japonica*		
	KBG		Wild (%)	KBG		Wild (%)
	Greenhouse (%)	Under forests (%)		Greenhouse (%)	Under forests (%)	
Natural pollination	9.4	–	15.0	2.0	–	12.6
Bagging	0	–	0	0	–	0
Artificial self-pollination	0	–	0	0	–	0
Artificial cross-pollination	93.3	63.9	–	61.5	34.5	–

Table 4 Chi-Quest values for the treatments under different environment

Treatment	Comparison	*H. aridicola*	*A. albus*	*S. parviflora*	*S. japonica*
Natural pollination	Wild * KIB	0.46 (NS)	34.73 (***)	1.30 (NS)	115.40 (***)
	June * August	19.70 (***)	–	–	–
Bagging	Wild * KIB	2.83 (NS)	0 (NS)	0 (NS)	0 (NS)
Artificial self-pollination	Wild * KIB	11.31 (***)	0 (NS)	0 (NS)	0 (NS)
Artificial cross-pollination	Wild * KIB	7.89 (*)	0.11 (NS)	–	–
	Greenhouse * under trees	–	–	4.61 (*)	4.03 (*)

NS non-significant

*0.05 > P > 0.01; **0.01 > P > 0.001; ***P < 0.001

47.0 ± 11.5%, suggesting that pollinators of this plant species occur in the wild but not at KBG. This is in accordance with the results of our pollinator observations. Besides that, the fruit set of artificial cross-pollination were 83.3% ($n = 30$) and 80.0% ($n = 30$) (Table 2), respectively, with no significant difference ($\chi^2 = 0.11$, $P > 0.05$) (Table 4) within both localities.

Stemona parviflora and *S. japonica*

The results of fruit sets of *S. parviflora* and *S. japonica* are shown in Tables 3 and 4. Both species did not set any fruit in bagging and by artificial self-pollination, independent on the locality. At KBG, the fruit set of artificial cross-pollination about *S. parviflora* in the greenhouse was 93.3% ($n = 15$) and 63.9% ($n = 36$) under trees. For *S. japonica* we assessed a fruit set of 61.5% ($n = 26$) in the greenhouse and 34.5% ($n = 29$) under trees. According to the obtained results, the fruit sets in the greenhouse was significantly higher than under trees for both species (*S. parviflora* ($\chi^2 = 4.61$, $P < 0.05$) and *S. japonica* ($\chi^2 = 4.03$, $P < 0.05$)). Besides that, both species showed clearly lower fruit sets (less than 10.0%) after natural pollination at KBG greenhouse (9.4% for *S. parviflora*: $n = 406$); 2.0% for *S. japonica*:$n = 1685$), indicating either a small number of pollinators or a low pollination efficiency. In addition, the fruit set of *S. parviflora* of natural pollination in the natural environment (15%, $n=40$) was higher than at KBG but without significant difference ($\chi^2=1.30$, $P>0.05$), while fruit set of *S. japonica* in natural environment (12.6%, $n=730$) showed a significant difference to KBG ($\chi^2=115.40$, $P<0.001$).

Discussion

From 1990s, Botanic Gardens Conservation International (BGCI) has been concentrated on the conservation of threatened plant species through botanical gardens and until now, botanic gardens have collected and conserved probably the majority of threatened plant species all over the world (Mounce et al. 2017). Though an increasing number of species conserved in botanical gardens, there are still many problems present. For example, protected species or areas are extremely imbalanced (Maunder et al. 2001; Mounce et al. 2017) and/or unknown or unclear germplasm provenances results in inbreeding or outbreeding depression (Zhang et al. 2010). Additionally, pollination and seed dispersal of threatened species ex situ conserved in botanical gardens were not considered. Xu et al. (2008) evaluated the growth and breeding situations of protected plants from eleven Chinese botanical gardens and found that only 38% of the investigated plant species flower regularly and only 64% of the flowering plants set fruits, which means in other words many threatened species are cultivated under inappropriate conditions. Unfortunately, there are no evaluation parameters defined which would allow whether threatened species cultivated in botanical gardens receive effective conservation (Maunder et al. 2001). Here, we firstly assessed pollination and mating systems, and reproductive successes for the four ex situ conserved threatened plant species. Based on our data, we firmly appeal to pay attention to the natural reproduction systems of plant species.

Pollination and seed dispersal are the most important processes in plant propagation (Calviño-Cancela et al. 2012; Neuschulz et al. 2016). Many species became endangered due to the failure of these two processes. For example, some orchid species became endangered because of limitations of appropriate pollinators (Martins and Johnson 2007; Swarts and Dixon 2009). For *H. aridicola*, *Xylocopa appendiculata* act as pollinators in August but no appropriate pollinators showed up during in June at KBG. This led to a significant difference in fruit set of natural pollination ($\chi^2=19.70$, $P<0.001$) (19.6% in June and 52.0% in August). Whilst for other three species, they all had few or even no pollinators at KBG resulting of no or low fruit sets. Hence, to secure the reproductive success of a plant species, hand-pollination or the introduction of appropriate pollinators when cultivated in botanical gardens. For species like the two *Stemona* species, few pollinators in their natural habitat may result from the low number of plant individuals in each population. Another reason may be the distance between neighbouring populations which hampers the attraction of a larger number of pollinators. This is a common phenomenon in the Allee effect as individual fitness decreases along with the population size and density (Stephens et al. 1999). Thus, reintroduction is the quickest and most effective method for the two species to enhance or augment existing populations for survival and conservation over the long run (Edward and Thomas 2007).

Mating systems are one of the most important biological factors affecting the genetic diversity and distribution pattern of plant population (Demauro 1993; Zhang 2004). According to our results of studying the mating systems of the four threatened species, *H. aridicola* is the only self-compatible species. But there is some pollen limitation in this species could be observed according to the pollen limitation index (L) was 0.44 in August at KBG and 0.62–0.72 in wild condition (Larson and Barrett 2000). Similar observations were already made in other species with mixed mating systems (Barrett 2003; Ashman et al. 2004). On one hand, autogamy can ensures the sexual reproduction without external forces (Takebayashi and Morrell 2001) but on the other side, continuous selfing may also lead to inbreeding depressions and further to a decreasing genetic diversity (Mccall et al. 1994; Cheptou et al. 2000). However, the other three studied species are all self-incompatible because there was no fruit set after artificial self-pollinated observable. Hence, they cannot propagate sexually unless they were visited by appropriate pollinators or hand cross-pollination was performed. Such species rely on their pollinators or external forces and therefore they are endangered to extinction when they loss their natural pollinators (Biesmeijer et al. 2006). Therefore, we claim that botanical gardens should pay more attention to such incompatible threatened species when ex situ conservation takes place in botanical gardens.

In this study, we used four threatened plant species to explore the restrictions for ex situ conservation of endangered species. Our results showed clearly, that much more attention should be paid on pollination biology as well as seed dispersal of threatened species under ex situ conditions. Plant species with extremely small populations in their natural habitat often show a reduction in their reproduction due to the lack of pollinators and/or seed dispersers. Thus, reintroduction of them may be an effective way to increase population sizes in order to facilitate the attraction of both, appropriate pollinators and seed dispersers.

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