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Pollination ecology, breeding system, and conservation of *Caldesia grandis* (Alismataceae), an endangered marsh plant in China

Wahiti Robert Gituru, Qing-Feng Wang*, Yong Wang, and You-Hao Guo

College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China

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Abstract. *Caldesia grandis* Samuelsson is an endangered wetland herb on the brink of extinction in the vast area of Mainland China, which holds close to one eighth of the world's vascular plant species. The pollination ecology and breeding system of *C. grandis* were investigated from three natural populations occurring in two wetlands near the top of Mangshan Mountain in Hunan Province, central China. The species is in flower from early July to late September with a peak in August. The process of flower anthesis in *C. grandis* begins at about 10.00 am and lasts about four-and-one-quarter h. The flowers lasted ca. 5.5 h. *Caldesia grandis* is self-compatible; however, autogamy resulted in lower seed set than geitonogamy and xenogamy as well as free pollination. Both pollen viability and the seed set in open-pollinated controls at the same site were typically high (65.44% and 71.78% respectively). Mean pollen: ovule ratios in the three populations ranged from 901.75 to 931.354. No seed germination was observed, either in the field or in laboratory experiments. Propagation is achieved through turions, which commonly occur in the inflorescences. Flies (Insecta; Diptera) were the most frequent visitors to the flowers of *C. grandis*; however, bees (Insecta; Hymenoptera) composed a larger proportion of effective pollinators. Existence analysis revealed a peak of mortality density in mid-May corresponding to an environmental sieve in the period following the establishment of plantlets. An updated diagnostic of the conservation status of *C. grandis* in China was formulated. The main threats to this species are catastrophic events, especially human impact activities (agriculture, hobbyists' collections, and infrastructure construction). Establishment of a monitoring program is proposed, and both *in-situ* and *ex-situ* strategies are recommended for effective conservation of *C. grandis*.

Keywords: Alismataceae; Breeding system; *Caldesia grandis*; Conservation; Endangered; Pollination; Rare endemic.

Introduction

The genus *Caldesia* Parl. (Alismataceae) includes three species, all of which are restricted to the Old World. *Caldesia grandis* Samuelsson is confined to mountainous bogs and marshes in Southeast Asia. In comparison to the other two species in the genus, *C. grandis* has received less attention from researchers (Lai, 1977). This is presumably due to its limited geographical distribution (Figure 1). Furthermore, it is a mountain species, occurring at altitudes of 1,000-1,500 m in localities that are frequently not easily accessible. *Caldesia grandis* has been found in China and the eastern Himalayas, but not in peninsular India (Cook, 1996), Japan or Myanmar. However, only a few records of localities of the species exist. In China the species has been listed as endangered (Fu and Jing, 1992). There is a dearth of information on *C. grandis* in the botanical lit-

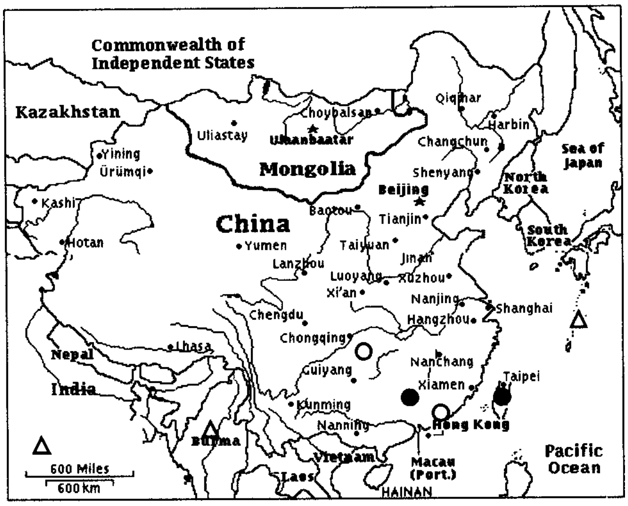

Figure 1. Global distribution of *C. grandis*: ●, Populations now surviving in China; ○, Location of recorded populations now decimated in China; △, Recorded populations outside China.

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*Corresponding author. Tel: +86-27-87682869; Fax: +86-27-87669560; E-mail: wangqf97@public.wh.hb.cn erature (Wang and Chen, 1997; Wang et al., 2001). The plight of *C. grandis* has not received adequate global attention in spite of the rarity and endangered condition of the species.

The study of reproductive biology is fundamental for systematic and evolutionary studies as well as conservation (Ornduff, 1969; Holsinger, 1991; Anderson, 1995). Conservation or restoration programs cannot be effective without an understanding of breeding systems and pollination (Weller, 1994). Considering the members of the Alismataceae as a whole, little is known of their reproductive biology (Flora-Lise, 1988). Observations of features, including floral morphology and phenology as well as pollination studies, can provide inferences into plant breeding systems. No information is available on the identity of the pollinators in *C. grandis* or on their relationship with the plant. In this study we investigated the habitat and the reproductive biology of *C. grandis*, including the pollination ecology, breeding systems, seed set, and pollen viability, with the objective of contributing to the knowledge of the biology of this rare plant. We also performed existence analysis to identify possible environmental sieves in the life history of *C. grandis* in the study populations. An updated diagnostic of *C. grandis* in China was formulated from the monitoring of its populations in the country from 1995 to 2001. It is hoped the information in this paper will provide a useful impetus for further study or conservation management of this endangered species.

Materials and Methods

Study Species

Caldesia grandis is a perennial (up to 30 cm tall), erect, glabrous marsh herb. Its leaves are radical with broadly elliptic to nearly suborbicular blades. The leaf bases are normally truncate and sometimes slightly cordate in smaller specimens. Scapes are few or solitary with panicles 40 to 60 cm high. In winter, the leaves become yellow and drop. In early spring, the young leaves have a whitish powdery appearance on their upper surfaces that disappears as the leaf matures. Our plants had many flowers, and vegetative buds were found among the flowers. Sometimes the plants bear whorls of turions instead of flowers on the inflorescence axis. After anthesis, turions are formed on the more or less prostrate stolon-like stem. Flowering from July to August, *C. grandis* in China has been reported from Hubei, Hunan, and Guangdong Provinces in the Mainland as well as from the island of Taiwan. *Caldesia parnassifolia* is a perennial wetland herb, exhibiting close similarities with *C. grandis* in growth habit and gross morphology. The two species, however, are readily distinguishable on closer examination. *Caldesia parnassifolia* has broad-elliptic leaves with an acute apex and a deeply cordate base. The lowest bracts subtending the pedicel in this species are up to 1 cm long. The flower has 6 stamens and 5-8 carpels. *Caldesia grandis* has reniform leaves with an emarginate apex and truncate base. The lowest bracts in this species are up to 2 cm long. The flower has 12-20 carpels and 9 stamens although specimens with up to 11 stamens have occasionally been found. In addition, all parts in *C. grandis* are coarser or larger than in C. *parnassifolia.*

Study Sites and Populations

The populations of *C. grandis* studied here are in Mangshan Nature Reserve (24°52'00"~25°03'12"N and 112°43'19"~113°00'10"E). The park has an area of 20.0 × 10^4 ha. The highest elevation in the area is 1,902 m. *Caldesia grandis* is found in three populations occurring in two marshes close to the center of the reserve. The populations were visited from June to November in 1999 and 2000. Data on the physico-chemical properties at the sites were obtained during the flowering period in 2000. Langpan Marsh, with two populations of *C. grandis* (LP1 and LP2), measures about 2 km^2 and has an average depth of 0.5~1 m. The marsh is densely covered with a thick mat comprised mainly of the peat moss *Sphagnum cuspidatum* Ehrh. Ex Hofm. and the fern *Cyclosorus acuminatus* (Houtt.) Nakai. A sluggish stream flows across the marsh, for the most part beneath the dense mat of vegetation. The shrub *Cephalanthus tetrandus* (Roxb.) Ridsd. et Bakh. occurs in the bog. Population LP1 occurs in a small area of open water measuring approximately 4 × 25 m. The other, much larger, population (LP2) is found 500 meters downstream and is separated by a patch of the bamboo *Sinarundinaria nitida* (Mitford) Nakai. The third population of *C. grandis* (GH1) is located in Guaihu Marsh, 8 km from Langpan Marsh. *Caldesia grandis* in this site is confined to the fringes of a centrally located area of open water measuring approximately 32 × 22 m, but also extends for about 5 meters along both sides of a small stream that flows into the marsh. About 60% of the population is shaded by a canopy *Viburnum macrocephalum* Fortune. During this study a population of the related species *Caldesia parnassifolia* (Bassi ex Linn.) Parl. was surveyed at Huli Marsh (26°5 1'79"N and 113°41'63"E, 50 m asl.) for the same characteristics for comparative purposes. Collections of *C. grandis* and *C. parnassifolia* from the study sites were sent to Wuhan University Herbarium (WH) for identification. Voucher specimens were deposited there.

Population Monitoring

All the localities of *C. grandis* recorded in the Flora of China and other localities reported since were visited at least three times a year by workers from our laboratory starting in August 1995 to September 2001. In the localities where extant populations of *C. grandis* were found, the number of individuals in the population was noted. Interviews were conducted with local people at all localities to gain insights into the human activities at the sites as well as any recent major changes to the habitats and populations.

Inflorescence Morphology, Flowering Phenology and Floral Biology

Inflorescence morphology was studied from flowering material obtained from all the four known extant populations of *C. grandis* in China. Population GH was used for studying flowering phenology. Ten individuals were chosen at random and tagged during the first day of flowering. From this day on, daily counts of open flowers were made, and opening sequences were determined for each inflorescence. Data were obtained for anthesis period, flower longevity, availability of pollen, nectar period, and fragrance production.

Floral Visitors

The behavior and movements of the insect visitors to the flowers as well as the number of visits to the flowers in the selected sites in the three populations of *C. grandis* were recorded in census periods of 15 min. The studies were conducted during the peak of flowering between 9.30 am and 11.30 am over eight non-consecutive days from 27th July to 29th August 2000. Studies on the population of *C. parnassifolia* were conducted between 30th August and 8th September 2000. The flights between plants and/or inflorescences were also recorded during these census periods. Twenty-minute intervals between the census periods were utilized for capturing specimens of the insect visitors using a hand held net. Pollen loads of the captured floral visitors were dispersed in a phosphate buffer 0.1 M and counted under a light microscope. Identification of the pollen grains was made by comparison with a reference collection at our laboratory.

Breeding Systems

Breeding experiments were conducted in population GH in the field and on materials that had been brought into cultivation and propagated at the aquatic garden at Wuhan University. Artificial pollination was effected by directly brushing the stigmas of the recipient flowers with stamens from the donors. Three phenomena, viz. autogamy, geitonogamy, and xenogamy, were tested by controlled crosses. Artificial self-pollination was conducted to test for active autogamy while spontaneous self-pollination (passive autogamy) was investigated by bagging intact flowers before they opened using pollination bags made of fine bridal veil. Artificial pollination, with pollen from other flowers of the same individual, was conducted to test for geitonogamy. Xenogamy was tested by artificial pollination with pollen from flowers of other individuals in the same population. To investigate parthenogenesis, buds were bagged one day before anthesis and emasculated before flower opening. Free pollinated open flowers, which were tagged but not bagged, were used as control. Interpopulational crosses between the three populations were also carried out. The difference in seed production was evaluated using the Kruskal-Wallis test.

Pollen Viability

Five newly opened anthers per plant were obtained from 10 plants each in populations LP1, LP2, GH and HL and brought into the laboratory. Pollen was extracted and germinated in dishes with sucrose solution concentrations of 0%, 5% and 10%. Dishes were left at room temperature (20°C) for a maximum of 24 h. Pollen grains were considered to have germinated when the pollen tube length was greater than or equal to pollen diameter. For both *C. grandis* and *C. parnassifolia* 10% sucrose solution was found to be optimal for pollen germination. Pollen was observed using a light microscope at X150 magnification.

Stigma Receptivity

Hand pollinations were performed and pollen tubes counted to establish the time of peak pollen germination. One hundred flowers on 20 plants were haphazardly chosen, emasculated before anther dehiscence, and covered with fine bridal veil to exclude visitors. Starting at 10.00 am, every 15 min, 5 flowers were uncovered, artificially pollinated with outcross pollen, and then re-covered. The flowers were left on the plants for 24 h, after which they were collected and fixed in Carnoy's solution. The pistils were excised, cleared in 0.8 N NaOH at 60°C, washed in deionized water, squashed on a microscope slide in a drop of 0.1% aniline blue in 0.1 N K_3PO_4, and observed for pollen tubes under a microscope using UV light following the methods outlined by Preston (1991).

Seed Germination

Achenes were gathered from plants in the field. Seeds were excised from the pericarp and scarified by removing a tiny distal fragment of the testa with a scalpel. They were then placed in sterile tap water in an incubator at 27°C and illuminated for 14 h each day (Wang et al., 2001).

Survival Analysis

Each year from March onwards in the spring of 1999 and 2000, a total of 12 sample quadrats, each measuring 1 m × 1 m, were selected in the high population density areas in the three populations of *C. grandis* in Mangshan Nature Reserve. Four of the plots (plots 1-4) were in population LP1 while four plots (plots 5-8) were in population LP2. The rest of the plots (plots 9-12) were in population GH. Four similar plots were also selected in the population of *C. parnassifolia* at Huli. A total of 174 young plants of *C. grandis* and 144 of *C. parnassifolia* were marked as soon as they emerged from the water surface in 1999. In the following year 186 young plants of *C. grandis* and 167 of *C. parnassifolia* were marked. In every quadrat the survival number, maximum length of leaf blade (measured along the midrib), number of petioles, and maximum length of petiole were measured every two weeks. In both years recordings were discontinued in November. The number of seeds was counted to assess fecundity. However, since all the observed young plants of *C. grandis* and most of those of *C. parnassifolia* were derived from turions rather than from seeds, the number of turions produced by an individual plant was also recorded. A life table for the populations was constructed using the experimental data obtained above, based on the theory of life history analysis (Muenchow, 1986). Calculation of mortality density function followed the methods of Ge and Chang (2001) using the formula:

$f(t_{mi}) = 1/h_i (1-P_i) P_{i-1} \cdots\cdots\cdots P_1$

Where, h_i = interval time between the measurements; t_{mi} = middle of the interval time; P = survival ratio. Mortality density function f(t) was therefore the mortality ratio per unit time.

Population Regeneration

Ten soil vouchers from the sediment in each population, 10 cm deep and 20 cm diameter, were obtained in mid- September 2000. The number of seeds in each sample was obtained. Where present, the seeds were tested for viability using tetrazolium chloride (Moore, 1973). A 1 m^2 plot was made in an area of high population density in each population. The number of seedlings and plantlets arising from turions was counted in the plots.

Results

Flowering Phenology and Floral Biology

Caldesia grandis in the study populations was in flower from the beginning of July to late September with a peak in August (Figure 2). The three populations surveyed maintained open flowers for a period of 84-91 days in each of the two years of observation. In 1999, population LP1 flowered a week earlier than the other two populations. However, in 2000 the three populations flowered at about the same time. Population HL of *C. parnassifolia* flowered from mid-July to early October in both 1999 and 2000. This population maintained open flowers for a period of 71-74 days in the two years of observation. In *C. grandis* the peduncle appears above the surface of the water in late June and elongates rapidly, achieving a length of up to 50 cm by early July. The lateral flowers on the panicle open first while the primary axis continues to grow. On each branch of the panicle the sequence of flower opening was anti-clockwise. The number of flowers formed on an inflorescence ranged from 36 to 186 (av. = 106.5; N = 60). (One individual obtained from population LP1 was observed with 280 flowers on an inflorescence.)

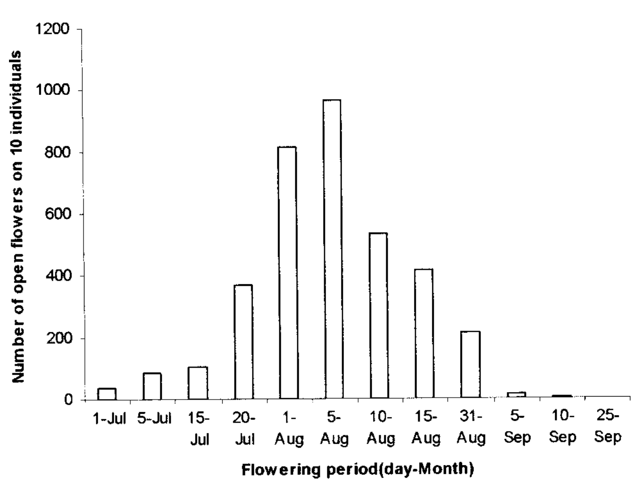

Figure 2. Flowering period of *C. grandis* in 2000 (GH population).

Anther dehiscence in *C. grandis* occurs soon after the start of flower opening at about 10.00 am, and the first insect visitors to the flowers can be observed at this time. Pollen and nectar are available for them. No odor was detected from the flowers. Stigmas were demonstrated to be receptive between 12:30 pm and 1:00 pm when the stigmatic surface assumed a pale whitish color. By 3:00 pm the anthers had changed from a yellowish-green to pale-brown and were virtually devoid of pollen. By this time pollen was observed on the stigma as well as on the petals.

Inflorescence and Floral Morphology

The inflorescences and flowers of the three populations of *C. grandis* had similar morphologies. Each individual produced a solitary inflorescence. The panicles were 40-60 cm high (av. 53.27; N=117). Regularly along the main axis were 5 to 7 (av. = 6.32; N=98) branches in whorls of 3. A total of 3 to 6 (av. = 4.33; N=108) flowers were borne in regular whorls of 3 on each branch. Along each branch there were 2 (occasionally 1) whorls. Each branch and the pedicel are sustained at the base by a membranous, acute, oblong-lanceolate bract. Whorls of turions were often borne instead of flowers on the inflorescence axis. These are vegetative lateral branches 1-1.59 cm long. Upon dissection these were found to consist of many imbricate, acute, lanceolate bracts. About 87% of the inflorescences we observed developed turions. Flowers were fasciculately 3-verticillate, regular and trimerous; sepals 3, green, ovate, deeply concave, 3.2-4.1 cm long, reflexed, persistent; petals 3, white, spathulate, 6-8 mm long, reflexed; stamens 12, 2.5 mm long, the anther basifixed; Carpels are 15-18 (av. =15.38; N=30) crowded in a small hemispherical head on a receptacle; nutlike, compressed, elliptic, 3 mm long (including the style), with longitudinal ribs; the style slender; 1.50-1.54 mm; Achenes have a long and more or less curved beak.

Floral Visitors

Of the 18 insect species regularly visiting the flowers of *C. grandis* and *C. parnassifolia* in the study populations, five Hymenoptera and two Diptera frequently made contact with the stigmas. Examination of captured specimens of these insects revealed copious amounts of *C. grandis* pollen attached to their bodies. These visitors were consequently deemed to be effective pollinators. Eight Diptera and three Hymenoptera made contact with the stigmas on four or less visits for every ten visits to the flower. These were considered to pollinate only occasionally while one Hymenoptera hardly ever pollinated (Table 1). The effective pollinator with the highest overall proportion of visits in the three populations of *C. grandis* was the Apidae species *Apis cerana* (Fabricius). In population GH, 32.2% of the visits were by effective pollinators as compared to 24.1% and 24.4% in populations LP1 and LP2, respectively. The proportion of effective pollinators to the population of *C. parnassifolia* was 22.8%. The most im-

Table 1. Insect visitation (%) to three populations of *C. grandis* and one population of *C. parnassifolia*.

Population	HL[d]	GH[a]	LP1[b]	LP2[c]	Pollination (rewards)[e]
INSECTS					
Diptera					
Syrphidae	**62.4**	**76.1**	**74.2**	**70.3**	
Episyrphus balteatus (De Geer)		37.1	37.2	36.7	Pollen +
Eristalinus lugens (Wiedeman)	28.2	9.9	9.5	12.0	Pollen +
Sphaerophoria indiana (Bigot)		9.7		5.1	Pollen; nectar ++
Sphegina sp.	5.1	3.0	7.4		Pollen +
Allobaccha apicalis (Laew)	1.4	5.0	6.2	4.8	Pollen +
Allobaccha rubilipennis (Austen)	5.1	6.2			Nectar +
Phytomia errans (Fabricius)	1.9				Nectar +
Eristalis arbustorum (Linnaeus)	19.7	5.2	10.8	11.7	Nectar +
Betasyvphus seravius (Wieclemann)	1.0				Nectar ++
Didea fasciata (MacGuart)			3.1		Pollen +
Hymenoptera	**37.6**	**23.9**	**25.8**	**29.7**	
Apidae					
Apis cerana (Fabricius)		14.4	12.5	14.6	Pollen; nectar ++
Halictidae					
Halictus simplex (Blüthgen)	4.0	8.1	7.6	4.7	Pollen; nectar ++
Halictus calceatus (Scopoli)		1.4	1.7	4.2	Pollen; nectar +
Halictus subopacus (Smith)			2.0		Pollen ++
Andrena sp.	6.0				Pollen; nectar +
Andrena parvula (Kirby)			2.0		Pollen; nectar ++
Andrena sp.	4.3			6.2	Pollen; nectar +
Halictus sp.	18.8				Pollen; nectar ++
Eumenidae					
Euodynerus trilobus (Fabricius)	4.5				Nectar 0

[a] GH (n=348, 30 h) 6th August - 15th August 2000.
[b] LP1 (n=55, 30 h), 27th July - 5th August 2000.
[c] LP2 (n=568, 30 h), 20th August - 29th August 2000.
[d] HL (n=574, 30 h), 30th August - 8th September 2000.
[e] ++ = effective pollinator; + = occasional pollinator; 0 = hardly ever pollinating.

portant group of insect visitors to the flowers of *C. grandis*, with respect to frequency of visits, was flies (Insecta, Diptera). The dipteran *Sphaerophoria indiana* (Bigot), an effective pollinator, was present in populations GH and LP2, but almost absent in population LP1 while *Andrena parvula* (Kirby), also an effective pollinator, was present only in population LP1.

The visiting behavior of bees entailed landing directly at the center of the flower followed by pollen collection, which was effected using the mouth parts, fore and middle legs, and the abdomen. *Halictus simplex* (Blüthgen) landed on the sepals (and occasionally on petals) and made its way to the center of the flower. The pollen collecting behavior of this species was similar to that of other bees except that it tended to tarry longer on a single flower (av. =38 sec.; N=100) than the other bees (av. =15 sec.; N=100). Except for *Halictus subopacus* (Smith), which foraged for pollen only, all bees foraged for nectar by probing the base of the sepals with their mouths. The hymenoptera *Euodynerus trilobus* (Fabricius) approached the nectaries by probing between the filaments after landing on the petals. It commonly remained on the flower for more than 60s and, unlike all the other visitors, never made contact with the stigma.

Breeding Systems

Table 2 summarizes the results of the breeding experiments. The general reproductive behavior of *C. grandis* was the same in the three populations. The parthenogenesis test failed to yield any seed. Flowers which had been bagged to exclude the presence of pollinators produced a mean of 2.3-2.9 seeds per flower, showing that more than 14% of the seed set can be achieved by autogamy. Active selfing led to a significant ($p<0.05$) increase in seed production up to a maximum of 49.45%. The best results in seed production ($p<0.05$) were obtained through free pollination at the field sites (up to 6-18 seeds/ flower, 52.03-71.78%). Contrary to the situation reported by Lai (1977) that achenes were “very rare” after anthesis, free pollination in the field site yielded a high fruit set. The population of *C. parnassifolia* at Huli, which served as a comparative external reference, displayed similar breeding system characteristics, including the absence of parthenogenesis and self-compatibility up to 35.82%. However, artificial self-pollination in this species yielded a lower seed set than in *C. grandis*. The seed set obtained from inter-population crosses between LP1, LP2, and GH was similar to that from within-population crosses (72.14-56.41%; Table 3).

Seed Germination, Pollen Production, Pollen Viability and P/O Ratios

No germination of *C. grandis* seeds was observed either in the field or in laboratory experiments. In contrast, 8.83% and 7.89% of the seeds of *C. parnassifolia* germinated under natural conditions in 1999 and 2000, respectively (Table 4). Fungal hyphae were commonly observed on the mature seeds of *C. grandis* in the field while these were still attached to the mother plant. Occasionally this fungal infestation was found on the flower buds. This phenomenon was not observed in *C. parnassifolia*.

The flowers of *C. grandis* have 10-12 stamens and mean pollen production ranges from 1,238.431 to 14,275.39 grains per flower, with 62.81-65.44% viability in the three populations. Comparison of the quantity of pollen produced by each of the anthers in a flower revealed no significant difference (Mean ± SE =1398.26±1308 grains, F7, 99=0.63, NS). The flowers have a pollen: ovule ratio (P/O) of 685.90-1148.99. The data on pollen production, viability and P/ O ratios are summarized in Table 5.

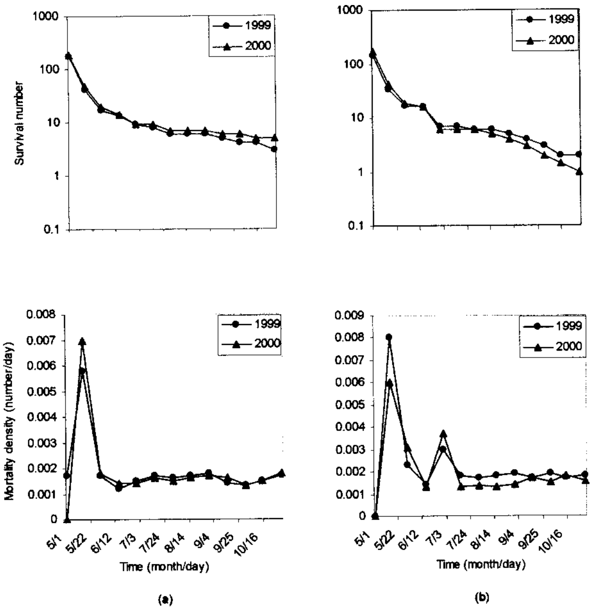

Figure 3. Survival curve and mortality density function of (a) *C. grandis* and (b) *C. parnassifolia* 1999-2000.

Table 2. Breeding system in three populations of *C. grandis* and one population of *C. parnassifolia*.

Treatment	Developed seeds					
	N	C_t	N_t	Range	M±SE	%
C. grandis (LP1 population)						
Free pollination (field site)	39	638	458	6-17	11.74±1.08	71.78
Free pollination (in cultivation)	40	643	403	3-16	10.07±1.17	62.67
Parthenogenesis	10	156	0	0	0	0
Passive autogamy	30	466	76	2-5	2.53±0.37	16.33
Active autogamy	29	492	219	3-11	7.56±0.82	44.51
Geitonogamy	38	628	300	1-15	7.89±0.73	47.77
Xenogamy	39	647	376	2-14	9.64±0.97	58.11
C. grandis (LP2 population)						
Free pollination (field site)	40	640	386	6-18	9.65±0.95	60.31
Free pollination (in cultivation)	38	589	364	3-17	9.57±1.59	61.79
Parthenogenesis	10	148	0	0	0	0
Passive autogamy	30	486	82	2-5	2.53±0.07	16.87
Active autogamy	32	546	270	3-10	8.43±0.83	49.45
Geitonogamy	29	447	209	1-14	7.2±0.96	46.75
Xenogamy	34	574	347	3-13	10.2±0.84	60.62
C. grandis (GH population)						
Free pollination (field site)	43	663	345	7-16	8.02±0.78	52.03
Free pollination (in cultivation)	39	629	393	2-14	10.07±0.79	62.48
Parthenogenesis	10	154	0	0	0	0
Passive autogamy	32	515	74	1-5	2.31±0.08	14.36
Active autogamy	29	429	210	4-12	7.24±0.87	48.95
Geitonogamy	30	510	223	1-14	7.43±0.91	43.73
Xenogamy	33	512	293	2-13	8.88±0.7	57.22
C. parnassifolia (HL population)						
Free pollination (field site)	38	285	169	0-7	4.45±0.17	59.4
Free pollination (in cultivation)	41	287	153	0-5	3.73±0.12	53.2
Parthenogenesis	10	78	0	0	0	0
Passive autogamy	48	312	56	0-3	1.17±0.14	17.95
Active autogamy	40	288	83	1-3	2.08±0.09	28.71
Geitonogamy	44	312	112	0-4	2.55±0.07	35.82
Xenogamy	42	243	76	1-4	1.81±0.04	31.3

N = sample size; C_t = total number of carpels; N_t = total number of seeds.

Survival Analysis

During both 1999 and 2000 in the populations of *C. grandis* mid-May was a period of noticeably elevated mortality density (Figure 3a), which corresponded with the period when the plantlets were young and their height above the water surface had not exceeded 5.5 cm. This constituted an environmental sieve in the period immediately following the establishment of the plantlets. Following the peak of mortality density, death of individuals occurred continuously; there were, however, no recognizable mortality density peaks. The population of *C. parnassifolia* at Huli also displayed a peak in mortality density in mid-May. In 2000 this population had a higher peak in mortality density than the *C. grandis* populations (Figure 3b).

Table 3. Interpopulation crosses between three populations of *C. grandis*, n=sample size and % of developed seeds in each case.

♀→ ♂↓	LP1	LP2	GH
LP1	n = 49 72.14	n = 56 62.11	n = 47 57.53
LP2	n = 36 62.13	n = 42 57.48	n = 34 65.61
GH	n = 53 56.41	n = 40 66.02	n = 47 57.37

Conservation Status

Information from the monitoring of the populations of *C. grandis* in China, which has been conducted by workers from our laboratory in the last several years, can be summarized in an updated diagnostic of the species as follows:

1. Number of individuals: visits to field sites in Conghua County of Guangdong Province and Hefeng County of Hubei Province, where *C. grandis* had been previously recorded, revealed that the populations have been decimated. The population of *C. grandis* in Yilan Marsh in northwest Taiwan had fallen below 50 individuals by January 2000. All three populations in Mangshan Nature Reserve recorded a decline in the number of individuals between August 2000 and August 2001. Population LP1 decreased by 11.36% while populations LP2 and LP3 decreased by 4.93% and 14.29%, respectively, over the same period.
2. Population regeneration: vegetative propagation from turions was achieved in pots. Estimation of seed bank reserve indicated that the soil vouchers from the study sites had viable seeds. Three soil vouchers from population LP2 showed 14, 16, and 12 seeds whereas the 7 remaining samples from the population had at least 5 seeds each. Two samples each from populations LP1 and GH showed 13 and 16 and 11 and 9 seeds, respectively. The remaining eighteen samples from the two populations had at least 6 viable seeds each.

Table 4. Life table of three populations of *C. grandis* and one population of *C. parnassifolia*.

Year	population	Seed percent germination	Number of seedlings	No. of young plants	Survival ratio	Fecundity (seed/plant)	Turions/plant
1999	LP1	0	-	36	59.41	112	56
	LP2	0	-	58	55.17	117	53
	GH	0	-	80	60.98	121	49
	HL	8.83	55	144	45.11	623	42
2000	LP1	0	-	41	53.1	128	55
	LP2	0	-	60	58.7	126	59
	GH	0	-	85	61.95	113	54
	HL	7.89	49	167	58.12	627	46

Table 5. Pollen production (Mean ± SE and range in parentheses), pollen viability (%) and pollen ovule ratio in 3 populations of *C. grandis* and one population of *C. parnassifolia*.

Population	Stamens	Pollen/anther	Pollen/flower	Viability	Ovules/flower	Pollen/ovule ratio
C. grandis						
GH	10.0361±0.02 (10-12)	1395.853±155.17 (15-1920)	12734.47±1310.2 (83-18730)	65.44613	14.411±2.903 (9-18)	931.354±217.644 (611-1143)
LP1	10.08824±0.4 (10-12)	1352.167±148.02 (28-1902)	12384.31±1315.8 (112-18891)	63.95197	14.364±2.91 (9-17)	928.4706±226.37 (635-997)
LP2	10.0161±0.2 (10-12)	1337.857±117.23 (36-1943)	12962.140±1313.25 (151-1870)	62.8142	14.4586±2.88 (9-18)	901.75±215.846 (702-1052)
C. parnassifolia						
HL	6.00	4256.971±275.4 (3593-4637)	25423.31±369.5 (21512-27690)	71.10979	6.371±1.02	3990.206±135.96 (3155-4532)

However, surprisingly, no seedlings were seen during visits to the three populations and a germination rate of 0% was obtained in laboratory experiments. Plantlets arising from turions were found in the three populations. A 1 m^2 plot in the area of high population density in population LP2 had 13 plantlets. Similar plots in populations LP1 and GH had 11 and 9 plantlets, respectively.

3. Competition and predation: In populations LP1 and GH competition for space was observed with clumps of the reed *Scirpus triangulatus* Roxb. (Cyperaceae). Competition with the same species for the services of the pollinators *Episyrphus balteatus* (De Geer) and *Apis cerana* (Fabricius) was observed in the three populations in Mangshan Nature Reserve. Mature leaves in the populations showed evidence of damage apparently caused by herbivore feeding, but no evidence of insect attack was found. Fungal growth was commonly observed on mature seeds and more rarely on flower buds.
4. Impact of human activities: Dry-weather roads have been constructed close to populations LP1 and GH, and a tea garden has been established <2 m from population GH. Population LP2 is crossed by a foot track. At the site of population GH locals were seen dumping truckloads of refuse from pigsties into the marsh.

Discussion

Flowering plants possess a wide array of morphological and physiological mechanisms that influence mating patterns, particularly the degree of self-fertilization (Richards, 1986; Eckert and Barrett, 1994). Temporal separation of male and female function within flowers (intrafloral dichogamy) is one of the most widespread morphological mechanisms and is found in >75% of cosexual angiosperm species (Bertin and Newman, 1993; Griffin et al., 2000). *Caldesia grandis* is protandrous; however a considerable overlap occurs between the periods of stigma receptivity and pollen release. The species is also self compatible. Self-compatibility among individuals of *C. grandis* is probably a derived condition, considering that its flowers are insect pollinated. Self-fertilization would be advantageous in establishment of the species in pioneer habitats such as the remote mountain bogs which *C. grandis* inhabits. Plants establishing themselves in pioneer habitats are likely do so following long-distance dispersal. “Bakers law” (Baker, 1955; 1967) suggests that the species most likely to become established after long distance dispersal are self-compatible. Rare plants are also likely to be self-compatible (Stebbins, 1970; Baker, 1959). Natural selection during repeated population bottlenecks may have resulted in self-compatibility in *C. grandis*.

The populations of *C. grandis* displayed only one peak of mortality density corresponding to an environmental sieve early in the life cycle of the plants. Thereafter, death of individuals continued throughout flowering and fruiting periods but at a much lower rate. This indicates a high likelihood for a young plant, once it is successfully established, to grow to maturity. The peak of mortality density in the populations during both years was also low (0.005-0.006). The reducing populations of *C. grandis* are therefore most likely as a result of habitat interference rather than a failure in plantlet recruitment. In all the three populations the peak of mortality density was higher in 2000 than in 1999; this may be related to the lower precipitation recorded in Mangshan Nature Reserve during 2000.

The abundance and efficiency of pollinators may affect floral evolution in plant populations by exerting different selective pressures (Grant, 1993; Galen, 1996). *Caldesia grandis* in the study sites receives a variety of visitors. Small bees and flies, known to be unspecialized pollinators (Opler et al., 1980; Muenchow, 1987), constitute the majority of the visitors to the flowers of *C. grandis*. Abundance and rarity of particular insect pollinators may account for the differences in the floral visitors to the three populations. The diptera *Sphaerophoria indiana* (Bigot), an effective pollinator, is active in populations GH and LP2 but is absent in population LP1 while the hymenoptera *Halictus subopacus* (Smith), another effective pollinator, is only found in population LP1. Except for *Halictus subopacus* (Smith), all the effective pollinators visiting the flowers of *C. grandis* extracted nectar. The energy investment in nectar production seems to be a very efficient pattern for the plant (Bloom et al., 1985). The bee *Apis cerana* (Fabricius) demonstrated oligolectic feeding (Linsley, 1958); it appeared during the blooming period of *C. grandis*, and microscopic examination indicated that *C. grandis* pollen was the only pollen attached to the bodies of specimens of this insect.

The failure of the seeds of *C. grandis* to germinate, probably due to the existence of highly specific germination requirements and the large numbers of plantlets arising from turions, indicates that vegetative propagation is the norm in this species. Limited sexual reproduction is a feature of many aquatic plants (Barrett et al., 1993). The tendency toward delayed germination is displayed in many species. In aquatic taxa, transport of vegetative fragments may lead more frequently to successful plant establishment than the dispersal of seed. Although no animals were observed feeding on the seeds of *C. grandis*, these may form a part of the diet of the several frutivorous bird species that abound in the fauna of the nature reserve. The occurrence of animal-mediated seed dispersal in the genus *Caldesia* has been noted by Cook (1990). Some Alismataceae seeds require scarification of the seed coat to break dormancy (Kaul, 1985). Invasion by fungal hyphae has been found to be a natural mechanism causing scarification of seeds in genus *Albizia* (Gogue and Emino, 1979). The presence of fungal growth that frequently occurred on the mature fruits and occasionally on flower buds of *C. grandis* requires further investigation. Furthermore, early in the flowering period in both *C. grandis* and *C. parnassifolia* there were indications of a preponderance of production of seed over that of turions. More studies are required into this phenomenon and its significance in the reproductive process in the two *Caldesia* species.

Prospects for Conservation in China

Caldesia grandis occurs in Southeast Asia, which is a global biodiversity "hotspot" (Myers et al., 2000) with exceptionally high species richness and a rapid rate of habitat destruction. China is a developing country with a large population, and the extraction of resources is very high (Zhu, 2000). The country also has one of the richest biodiversities in the world with more than 30,000 species of higher plants, making up 10% of the world total (Zhang, 1998). The expansion of agriculture and urbanization is causing rapid destruction of natural habitats. Considering that China alone houses about one-eighth of the world's flora, and that it is known only sketchily from a floristic point of view (Brach, 1996), the implications of such habitat loss to plant conservation are serious. *Caldesia grandis* was believed to be extinct in Mainland China until a finding was made by the present authors on Mangshan Mountain in 1995. Because the distribution area of *C. grandis* is narrow and the species is rare, plant collectors and hobbyists frequently collect specimens from the wild. Fortunately Mangshan Nature Reserve has so far protected the populations from such collection. Elsewhere, in the Yilan Wetlands of Taiwan the activities of collectors threaten to render the species extinct on the island (Lin, 2000). There is an urgent need to pay more attention to minimizing anthropogenic disturbances that can potentially result in extinction of the species. Care needs to be taken to avoid complacence regarding *C. parnassifolia*, which is presently not endangered, since the species is known to have become extinct in countries like Bulgaria (Dimitra et al., 2000). No current measures exist for conservation of the wild populations of *C. grandis* in China. We recommend that, as a preliminary step, a monitoring program be built for *C. grandis* in the country, including extensive exploration of favorable habitats, while looking for additional natural populations. *In -situ* conservation is recommended in order to preserve the existing genetic diversity. In addition, *ex-situ* cultivation at various sites should be undertaken to enhance recovery of genetic diversity.

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瀕危植物寬葉澤苔草（*Caldesia grandis* Samuelsson）的繁育系統、傳粉生態學和保護生物學研究

Wahiti Robert Gituru　王青鋒　王勇　郭友好

武漢大學生命科學學院

中國是世界上生物多樣性最豐富的地區之一，擁有世界上近 1/8 的維管植物物種。本文通過野外調查和實驗，對瀕危植物寬葉澤苔草在中國大陸目前僅存的三個自然居群的傳粉生態學和繁育系統進行了研究，並進行了生存力分析。寬葉澤苔草的花期爲七月上旬至九月下旬，八月爲其高峰期。單花的開放始於上午10點鐘，持續約四小時15分鐘。該種自交親和，但自花授粉的結實率與自由傳粉相似，低於同株和異株異花授粉的結實率，在同一地點進行的開放式傳粉控制試驗顯示花粉活力和結實率均較高，分別爲65.44%和71.78%。寬葉澤苔草主要通過發生在花序上的珠芽進行繁殖。花期最頻繁的訪花者爲蠅類，但有效傳粉的主體則爲蜂類。生存力分析顯示寬葉澤苔草死亡率的高峰出現在五月中旬，這與此時的環境篩選有關，在此之後倖存的幼苗開始定居。對該物種的威脅主要來自於災難性事件，尤其是人類活動的干擾（如農業生產、植物愛好者的採集和基礎設施建設等）。本文認爲建立有效的監測網絡以及就地和遷地保護是保護寬葉澤苔草和其他瀕危水生植物的有效途徑。

關鍵詞：澤瀉科；寬葉澤苔草；瀕危；傳粉。