
CRYPTIC DIOECY IN *NYSSA*
YUNNANENSIS (NYSSACEAE), A
CRITICALLY ENDANGERED
SPECIES FROM TROPICAL
EASTERN ASIA¹

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ABSTRACT

Nyssa yunnanensis W. Q. Yin ex H. N. Qin & Phengklai (Nyssaceae) is a critically endangered range-restricted tree species known from only three small populations in a tropical forest area of southern Yunnan Province, southwestern China. Two types of individuals occur, one bearing staminate flowers and the other with morphologically perfect flowers that produce both pollen and fruit, suggesting an androdioecious breeding system. Field and laboratory studies conducted between 2004 and 2007 indicate, however, that *N. yunnanensis* is functionally dioecious: pollen from the morphologically perfect flowers is inaperturate and inviable, rendering the trees that bear these flowers functionally female. Field observations showed that the staminate flowers opened 10 to 15 days earlier than the protogynous female flowers but that flowering ceased at nearly the same time in both sexes. Thirty-six species of insects were observed and collected visiting the flowers of *N. yunnanensis*, four of which served as effective pollinators. Breeding system experiments demonstrated that the predominantly entomophilous pollination system is supplemented by anemophily and further indicated that *N. yunnanensis* is xenogamous and does not appear to exhibit parthenogenesis. The average sex ratio of individuals within the three populations was female-biased (0.57:1), but the ratio among flowers was male-biased (2.56:1) because flower production was higher in males. The 37 known trees of *N. yunnanensis* are likely the remnants of a once more widespread, abundant species that has been heavily impacted by human disturbance, a fate shared with many other threatened Southeast Asian taxa whose continued survival will require dedicated conservation efforts informed by a detailed understanding of population structure and reproductive biology.

Key words: Androdioecy, critically endangered, cryptic dioecy, floral phenology, IUCN Red List, *Nyssa*, *Nyssa yunnanensis*, Nyssaceae, pollen viability.

The existence of dioecism in flowering plants has been universally acknowledged since Darwin (1877), yet dioecy is still poorly understood from both an ecologic and evolutionary point of view (Bawa & Opler, 1975). The reason for this may be the relatively low proportion of dioecious taxa (Yamplosky & Yamplosky, 1922), although Ashton (1969) and Bawa and Opler (1975) reported a large number of dioecious tree species in tropical forest and dioecy appears to be favored in island environments (Baker & Cox, 1984; Sakai & Weller, 1999; Carpenter et al., 2003). So far, however, relatively few evolutionary studies have been

conducted on tropical forest trees from eastern Asia (e.g., Ashton, 1969, 1977).

The use of a functional perspective to study plant sexual systems has greatly advanced our understanding of sexual strategies in these organisms and has paved the way for testing relevant evolutionary hypotheses on the phenotypic distribution of sex allocations in populations (Lloyd, 1980; Mayer & Charlesworth, 1991; Sakai & Weller, 1999; Delph & Wolf, 2004; Dunthorn, 2004; Pannell, 2005). Dioecious species may vary in their sexual expression resulting in androdioecy, gynodioecy, trioecy, or

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subdioecy (Sakai & Weller, 1999). Mayer and Charlesworth (1991) defined cryptic or functional dioecy as a breeding system that has unisexual morphs, at least one of which appears to have perfect, hermaphroditic flowers. Flowers of such morphs in cryptically dioecious taxa retain nonfunctional organs, either as a gynoecium in functionally staminate flowers or an androecium in functionally pistillate ones. The sex ratio of functionally dioecious species is expected to be 1:1 in populations (Lloyd & Webb, 1977), although a biased sex ratio is in fact often observed (e.g., Melampy & Howe, 1977; Mayer & Charlesworth, 1991; María & Ramón, 1995; Queenborough et al., 2007).

Nyssa L. (Nyssaceae) comprises about 13 species, with four in North America, one in Costa Rica, one ranging from India to Indonesia, and seven in China (including six endemic species) (Qin & Phengklai, 2007). The genus exhibits a disjunct distribution between eastern Asia and North America (Eyde, 1966, 1988; Wu & Fan, 1977; Fang et al., 1983; Wen & Stuessy, 1993; Wu et al., 2003) and has a rich fossil record in the Tertiary of the Northern Hemisphere (Eyde, 1963). The reproductive system of *Nyssa* has often been described to be polygamodioecious (e.g., Wangerin, 1910; Eyde, 1963; Wu & Fan, 1977; Wen & Stuessy, 1993; Qin & Phengklai, 2007), although it has not been documented clearly for most species. Wangerin (1910) presumed that *Nyssa* species were pollinated by wind and insects. Cipollini and Stiles (1991) and Batra (1999) studied the cost of reproduction and the behavior of native flower-visiting bees in the North American species *N. sylvatica* Marshall. Both studies indicated that *N. sylvatica* is dioecious and documented native bees as its pollinators. *Nyssa aquatica* L., another North American species, is also dioecious and may be pollinated by both insects and wind (Shea et al., 1993), but the pollination biology and breeding systems of Asian members of the genus are still poorly known.

Nyssa yunnanensis W. Q. Yin ex H. N. Qin & Phengklai was originally (and invalidly) described by Wu and Fan (1977) based on two collections, one bearing only staminate flowers and the other in fruit. Wen and Stuessy (1993) reconstructed the phylogeny of *Nyssa* using 18 morphological characters and regarded *N. yunnanensis* and *N. javanica* (Blume) Wangerin as closely related taxa with the two species possessing capitate male inflorescences. Because the morphology of *N. yunnanensis* was poorly documented, Wen and Stuessy (1993) treated it as belonging to the *N. javanica* complex and used *N. javanica* to represent this presumed lineage in their phylogenetic analysis in an attempt to reduce the impact of missing data. Based on comparisons of herbarium specimens

and field observations, we found that *N. yunnanensis* differs from *N. javanica* in bearing thicker leathery leaves, but both appeared to be morphologically androdioecious, with structurally male and hermaphroditic flowers borne on different individuals coexisting in the same population (Sun & Zhang, 2007). Androdioecy is a rare breeding system in angiosperms (Darwin, 1877; Lloyd, 1975; Charlesworth & Charlesworth, 1978; Bawa & Beach, 1981; Ross, 1982; Charlesworth, 1984; Liston et al., 1990; Fritsch & Rieseberg, 1992; Pannell, 2002), and many reported instances have proven to be functionally dioecious when examined in detail, with the morphologically perfect flowers in fact being functionally female (e.g., Charlesworth, 1984; Anderson & Symon, 1989; Schlessman et al., 1990; Mayer & Charlesworth, 1991).

Nyssa yunnanensis is a canopy tree species whose range is located at the northern edge of the East Asian tropical zone. It is confined to mountainous bogs and marshes in southern Yunnan Province, China (Fu, 1989), and is seriously threatened (Fu, 1992), having been listed as Critically Endangered (CR) in 2004 based on the IUCN Red List criteria (IUCN, 2008) and proposed for transfer from Rank III to Rank I protection in China (Wang & Xie, 2004). Understanding the reproductive biology of highly threatened species such as *N. yunnanensis* is important for developing conservation strategies, especially when they are known only from very few populations (see Falk & Holsinger, 1991; Bernardello et al., 1999).

In this study, we documented the floral phenology, pollen morphology and germination, sex ratio, pollination biology, and mating system of *Nyssa yunnanensis* to address the following questions: (1) Is *N. yunnanensis* functionally dioecious? (2) If so, what is its sex ratio? (3) Is it pollinated by wind, insects, or both? (4) Can any relationships be detected among the breeding system, sex ratio, and the rarity of *N. yunnanensis*?

MATERIAL AND METHODS

STUDY SPECIES

Nyssa yunnanensis is a critically endangered wetland tree up to 30 m in height. It has small green-yellow sessile flowers (ca. 2 mm long) arranged in capitulae less than 5 mm wide (Sun & Zhang, 2007). Its flowers, which are of two types, structurally male and perfect, secrete abundant nectar with an applelike fragrance from the surface of the ovary disc. The single-seeded drupes have a low germination rate, and no seedlings have been observed in natural populations (Sun et al., 2007). *Nyssa yunnanensis*

often grows in association with species of *Quercus* L., *Juglans* L., *Persea* Mill., and *Laurus* L., particularly in riparian vegetation along rivers (Fang et al., 1983).

STUDY SITE

Nyssa yunnanensis is known from only three populations at Puwen Tropical Forest Station (101°06'E, 22°25'N) in southern Yunnan, one along a stream at 842 m (hereafter referred to as the Stream population), one around a well at 837 m (the Well population), and the third at ca. 900 m adjacent to an old dam (the Dam population). The size of these three populations is small, with 14, 9, and 14 individuals, respectively. Field investigations were conducted from 2004 to 2007. Experiments were carried out continuously on two trees bearing staminate flowers and three trees with morphologically perfect flowers (all others were so tall, reaching to almost 30 m in height, as to make it difficult or impossible to access flowers on a regular basis). In order to assess sex ratios, we did, however, have local people climb all trees in each of the three populations to collect specimens.

FLORAL PHENOLOGY

Floral phenology was studied in the wild populations of *Nyssa yunnanensis* in 2006. Two tagged male trees were monitored to record information of phenological phases, such as morphological changes, flowering period, flower life span, anther dehiscence, nectar production, odor, and fruiting. Data were recorded from a total of 40 fertile branches bearing 197 inflorescences and a total of 2811 flowers. The same types of information were collected from three trees bearing morphologically perfect flowers with a total of 40 branches bearing 82 inflorescences and 590 flowers. Flower longevity was assessed by daily observation of 30 flowers randomly sampled and tagged before anthesis. Information on other stages of flowering and fruiting was obtained by weekly observations.

POLLEN VIABILITY AND STIGMATIC RECEPTIVITY

Pollen from both male and morphologically perfect flowers was examined with a light microscope (10×) and a scanning electron microscope (KYKY-1000B; Science Apparatus Co. of the Chinese Academy of Sciences, Beijing, China). Pollen viability was estimated *in vitro* by recording pollen germination in a gradient series of sucrose solutions (0.5%, 1%, 2%, 5%, and 10%) with distilled water as the control (Hu, 1993). Mature anthers were identified by color

change, 10 of which were collected from male and from morphologically perfect flowers, respectively, and examined under the light microscope once per hour, with each experiment repeated five times. The inflorescences were collected, taken to the lab, and placed in a culture dish containing water to test pollen longevity. Observations were made every three hours. The data were analyzed using the software package SPSS 11.0 (SPSS Inc., Chicago, Illinois, U.S.A.). Stigmatic receptivity was checked by examining changes in style color and shape, and then verified by diamino benzidine (DAB) (Dafni, 1992).

SEX RATIO WITHIN POPULATION AND AMONG FLOWERS

The within-population sex ratio of individual trees was determined for all 37 individuals at the three known sites based on observations made over the three years from 2005 to 2007. For each tree, a visual inspection of 30 to 100 flowers from approximately five inflorescences allowed us to determine its sexual status.

In order to determine whether *Nyssa yunnanensis* is functionally dioecious, consideration had to be given to the relative number of staminate and morphologically perfect flowers (the latter potentially being functionally female) in the populations (see Opler & Bawa, 1978; Webb & Lloyd, 1980). We therefore estimated the total within-population ratio of structurally staminate to perfect flowers (hereafter referred to as the population flower ratio [PFR]) according to a modified version of the method proposed by Opler and Bawa (1978). The PFR was calculated by multiplying the ratios of morphologically staminate and perfect flowers per inflorescence (the flowers per inflorescence ratio [FR]) by the ratios of inflorescences bearing structurally staminate and perfect flowers per branch (the inflorescence ratio [IR]), and then multiplying this in turn by the ratios of structurally staminate and perfect branches per tree (the branch ratio [BR]) and by the population sex ratios (PR). The resultant product, which can be expressed by the following formula, $PFR = FR \times IR \times BR \times PR$, provides an estimate of the ratio of total number of staminate to total morphologically perfect flowers in the three known populations of *N. yunnanensis*. For our estimate of the ratio of flowers per inflorescence, we examined all 37 individuals in the three populations and randomly selected 50 staminate inflorescences and 50 morphologically perfect ones to assess the FR, 40 branches from trees with staminate flowers and 40 branches from individuals with morphologically perfect flowers to determine the IR, and finally five trees with staminate flowers and five with morphologically perfect flowers to calculate

the BR. Sex ratios are consistently presented as male:female, with the second term always given as unity (1) such that ratios in which the first term is less than one indicate a female-biased sex ratio and those with the first term greater than one indicate a male-biased ratio.

FLORAL VISITORS

Floral visitors were recorded in the field at flowering time between 07:00 and 23:00 during 11 days between 15 and 25 March 2006, for a total of 176 hours of observation. Pollinator behavior and movement between the male and the morphologically perfect flowers were documented by photographs. Insect visitors were captured and brought to the laboratory of the Puwen Tropical Forest Station for identification and for further examination under the light microscope to detect any presence of pollen. Voucher insect specimens were deposited at the Kunming Institute of Botany.

MATING SYSTEM

To test whether individuals with morphologically perfect flowers produced viable pollen and were capable of both female and male function, an experimental protocol was used during the 2006 and 2007 flowering seasons involving a control and seven treatments, as follows: flowers were (1) untreated to serve as the control; (2) bagged to assess whether anthers on morphologically perfect flowers are capable of self-pollination in the absence of pollinators; (3) emasculated and bagged to investigate possible parthenogenesis; (4) emasculated and artificially self-pollinated to test for intra-flower self-compatibility; (5) emasculated and artificially pollinated with pollen from another morphologically perfect flower from the same individual to test for inter-flower self-compatibility; (6) emasculated and artificially cross-pollinated with pollen from a male flower to test xenogamy and to assess whether gene flow is possible between the plants with staminate flowers and those with morphologically perfect flowers; (7) emasculated and netted to test wind pollination; and (8) directly emasculated to test the function of pollen on the morphologically perfect flower by comparison with the results of the control group. Bags of MF tracing paper (approximately 20 × 20 cm in size and reinforced with nylon fiber), tested to make sure they did not allow the penetration of airborne pollen, were placed on immature inflorescences at the beginning of March and removed in mid-April when all flowers had withered. Artificial pollination was conducted by directly brushing the

stigmas of the recipient flowers with stamens from the donor. As indicated above, because many trees are too tall to carry out the various treatments, just three individuals with morphologically perfect flowers were used in 2006 and one in 2007 as the female parent, and two individuals were used as pollen donors. In general, we chose flowers from the highest part of these trees to serve as controls; flowers lower down were used to carry out the other treatments because they were easier to access and manipulate for artificial pollination. It should be noted that light intensity increases toward the top of these large trees. Mature fruits were collected from the control and the treated flowers in August.

RESULTS

FLOWER PHENOLOGY

Anthesis in *Nyssa yunnanensis* extended from February to April (Fig. 1). Staminate flowers opened 10 to 15 days earlier than the morphologically perfect ones, but flowering ceased at nearly the same time. The staminate flowers had five to seven petals, 10 to 14 stamens, and a central disc that secreted abundant nectar (Fig. 2A). The morphologically perfect flowers had four to six petals, five to seven stamens, a nectar disc that also produced abundant nectar, and a bifid style (Fig. 2B). The stamens in the staminate and the morphologically perfect flowers were similar in morphology, but they were more numerous in the staminate flowers and were arranged equidistant around the disc, forming two alternating whorls with filaments of different length (Fig. 3). Growth and the order of dehiscence of the anthers on the longer stamens were recorded on staminate flowers with five petals and 10 stamens. The order of dehiscence (shown on the left of Fig. 3) was sequentially from anthers number 1 to 5. By contrast, the short anthers in the staminate flowers did not develop and dehisce in a regular order. The stamens of the morphologically perfect flowers are equal in length, although they too can be divided into two groups. The first group includes most of the stamens, which develop early and are inserted around the disc. The second group, comprising just one or two stamens, is positioned directly on the disc and develops well after the flower opens. We refer to members of this second group as anaphase stamens (Fig. 3, right).

The life span of staminate flowers was 10 to 14 days, whereas that of the morphologically perfect flowers was slightly shorter, from 11 to 13 days. The anthers of the long stamens in staminate flowers dehisced and dispersed pollen four days after the opening of the flower; those on the short stamens

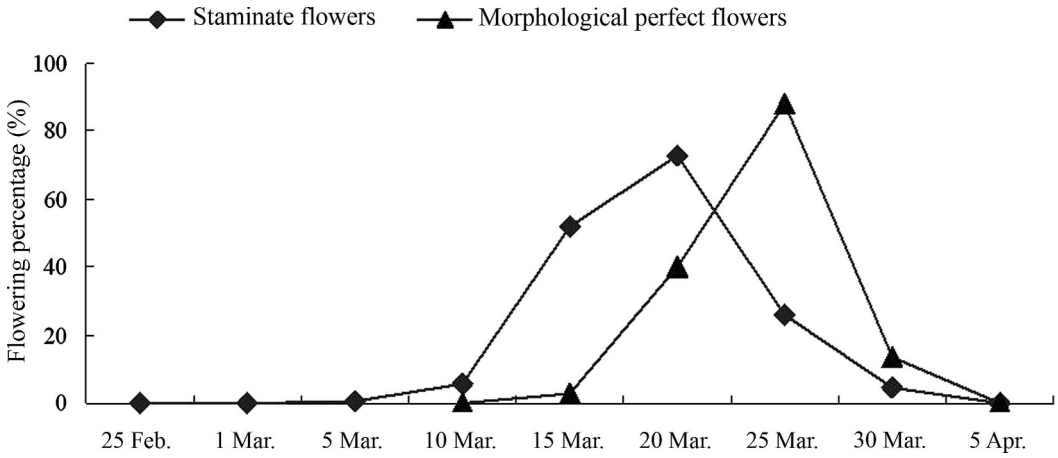


Figure 1. The flowering period of *Nyssa yunnanensis* W. Q. Yin ex H. N. Qin & Phengkklai in the studied population in 2006.

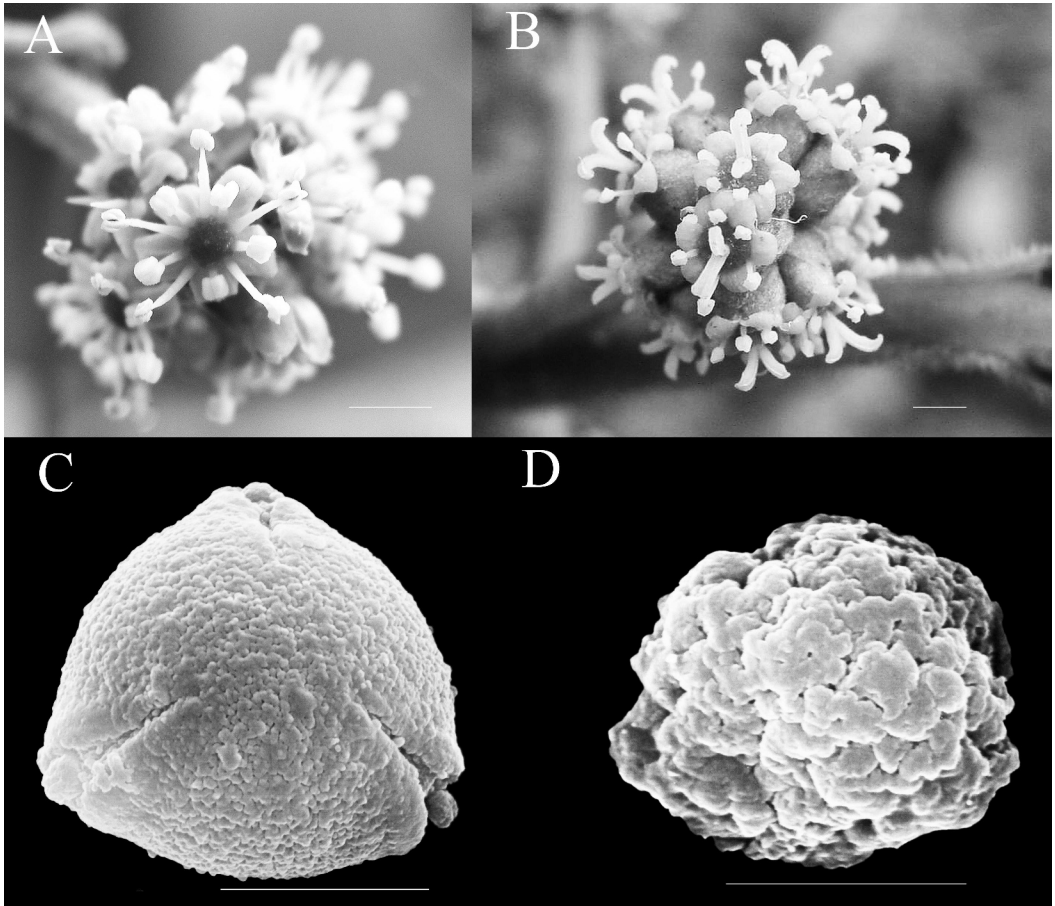


Figure 2. SEM images of flowers and pollen of *Nyssa yunnanensis* W. Q. Yin ex H. N. Qin & Phengkklai. —A. Staminate flowers. —B. Morphologically perfect flowers. —C. Pollen grain of staminate flower. —D. Pollen grain of morphologically perfect flower. Scale bars: A, B = 1 mm; C, D = 10 μ m.

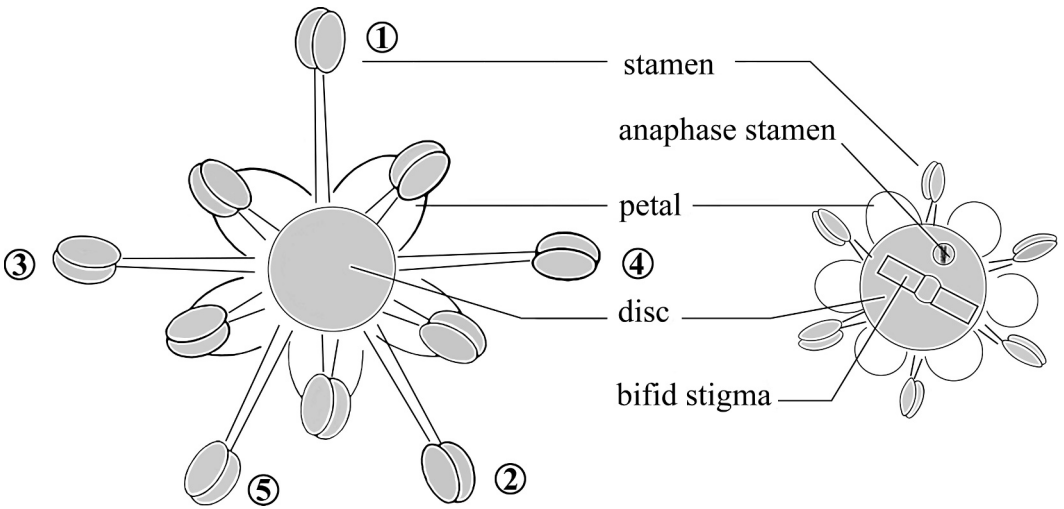


Figure 3. Flower structure of *Nyssa yunnanensis* W. Q. Yin ex H. N. Qin & Phengklai, showing staminate flower (left) and morphologically perfect flower (right) (the actual number of petals varies from five to seven in the male flower and four to six in the morphologically perfect flower).

released pollen one or two days later, after which the filaments of the short stamens elongated to reach the length of the long stamens. On the first day when the morphologically perfect flowers opened, the style was green and undivided, but after three or four days it began to become evidently bifid and the color changed from green to white, a process that took about five or six days to complete. The anthers of the morphologically perfect flowers dehisced seven or eight days after the opening of the flower. Nectar secretion began after the flower had been open two or three days and ceased when the stamens and style became brown.

POLLEN VIABILITY, STIGMATIC RECEPTIVITY, AND FLORAL SEXUAL FUNCTION

The diameter of the pollen grains from staminate flowers ranged from 25–30 μm . They were tricolporate and triangular in polar view, with scabrate and reticulate sculpturing (Fig. 2C). Morphologically perfect flowers produced only inaperturate pollen grains, which were circular in polar view (Fig. 2D). The pollen from staminate flowers germinated in a gradient series of sucrose solutions ranging from 0%–10% (Fig. 4), with the 0.5% sucrose solution yielding the best result (up to 97.4%, F5 [Levene F statistic of the

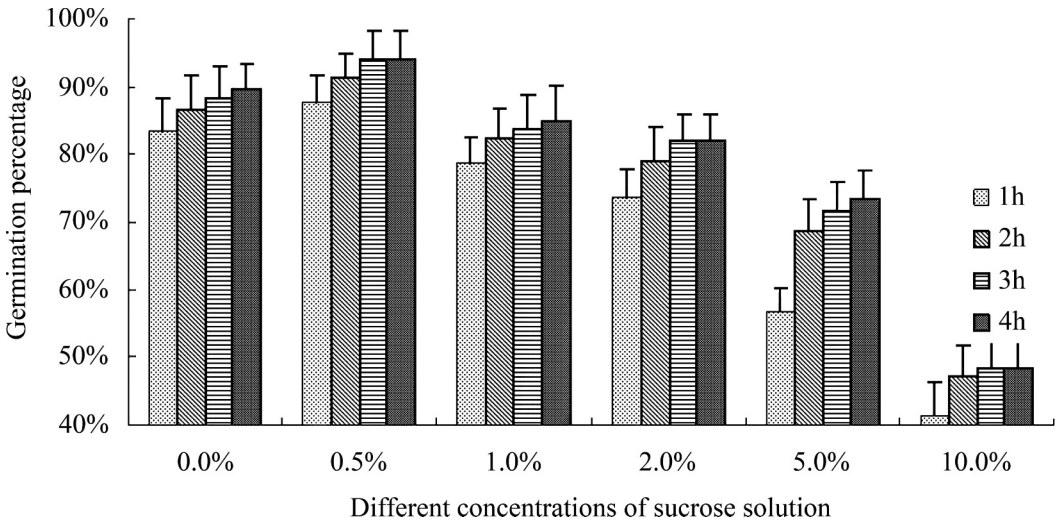


Figure 4. Pollen germination rate of *Nyssa yunnanensis* W. Q. Yin ex H. N. Qin & Phengklai in different concentrations of sucrose solution and different culture times.

Table 1. Sex ratio and flower sex ratio in three populations of *Nyssa yunnanensis*.

	Sex ratio (PR)			Flower sex ratio (PFR)		
	SP (M:H:J)	WP (M:H:J)	DP (M:H:J)	FR (n = 50)	IR (n = 40)	BR (n = 5)
Ratio	6:6:2	0:4:5	2:4:8	1.94:1	2.39:1	0.97:1
Average of three populations	0.57:1			2.56:1		

BR, branches per tree; DP, Dam population; FR, flowers per inflorescence ratios (staminate/morphologically perfect); H, morphologically perfect; IR, inflorescences per branches ratio (staminate/morphologically perfect); J, juvenile; M, staminate; PFR, population flower sex ratio (staminate/morphologically perfect) ($PFR = FR \times IR \times BR \times PR$); PR, sex ratio of plants in population; SP, Stream population; WP, Well population.

five samples] = 1.654, $P < 0.05$, using distilled water as the control). The pollen germination rate increased more or less regularly with increased cultivation time, although a slight change occurred after three hours. By contrast, no pollen from the morphologically perfect flowers germinated, regardless of sucrose concentration and culture time, indicating that these flowers are functionally female.

In morphologically perfect flowers, as indicated above, the style became receptive after changing color from green to white and then turned brown following receptivity. The stigma was receptive before dehiscence of the first group of anthers, so the morphologically perfect (and functionally female) flowers were thus protogynous.

SEX RATIO WITHIN POPULATIONS AND AMONG FLOWERS

Three populations of *Nyssa yunnanensis* are present at the Puwen Tropical Forest Station: the Stream population, the Well population, and the Dam population. When juvenile individuals were excluded, the ratio of adult male to female trees was 1:1 in the Stream population (six male, six female, and two juvenile trees), 0:1 in the Well population (zero males, four females, and five juveniles), and 1:2 in the Dam population (two males, four females, and eight juveniles) (Table 1). When all 37 individuals of *N. yunnanensis* known from the study area were considered, the overall sex ratio was 0.57:1, showing a clear female bias.

The $FR = 1.94:1$ (number of flowers per male inflorescence = 14.66 ± 3.27 , $n = 50$, $P \leq 0.05$; number of flowers per female inflorescence = 7.56 ± 1.34 , $n = 50$, $P \leq 0.01$). The $IR = 2.39:1$ (number of inflorescences per male branch = 4.90 ± 2.11 , $n = 40$, $P \leq 0.01$; number of inflorescences per female branch = 2.05 ± 1.01 , $n = 40$, $P \leq 0.01$). The $BR = 0.97:1$ (number of branches per male tree = 18.80 ± 2.86 , $n = 5$, $P \leq 0.01$; number of branches per female tree = 19.40 ± 2.51 , $n = 5$, $P \leq 0.05$). Finally, the overall $PFR = 1.94:1 \times 2.39:1 \times 0.97:1 \times 0.57:1 = 2.56:1$, which suggests a male-biased flower sex ratio.

FLORAL VISITORS

Thirty-six insect species belonging to five families and 25 genera were collected as visitors of *Nyssa yunnanensis* flowers. Twenty species were observed on male and/or functionally female flowers (Appendix 1). Because male flowers opened before female flowers, insects were at first observed and captured visiting the former. Most insects visited flowers exclusively for nectar, and only four species were observed to carry pollen on their body and/or legs and could therefore be considered potential pollinators: *Chrysomyia megacephala* Fabr. (a fly), *Apis cerana* Fabr. (a bee), Eurytomidae sp., and *Praestochrysis* Linsenmaier sp. (both chalcidoids). Pollen grains were also noted on the bodies of *Polistes* Latreille sp. and *Mutilla marginata* Baer, but these species only visited flowers occasionally and were thus not considered to be effective pollinators. Other visiting insects were observed, including syrphids, beetles, mosquitoes, and small moths, all apparently for nectar, but they neither carried pollen nor remained in the population of *Nyssa* for more than a short time, suggesting that they played little or no role in pollination.

MATING SYSTEMS

The results of the breeding experiments (Table 2) showed that fruit set from untreated flowers was higher than that from the other treatments in both of the years studied (49% in 2006 and 45% in 2007). Fruit set was zero for the following treatments: directly bagged, emasculated and bagged, emasculated and artificially self-pollinated with pollen from the same flower, and emasculated and artificially self-pollinated with pollen from another functionally female flower on the same tree. In functionally female flowers that were emasculated and artificially cross-pollinated with pollen from a staminate flower, fruit set at a rate only slightly lower than that in untreated flowers (47% in 2006 and 43% in 2007). Fruit set in functionally female flowers that were emasculated and netted was only 13% in 2006 and 15% in 2007, suggesting that

Table 2. Number of flowers and percentage fruit set in control and treated flowers of *Nyssa yunnanensis* over a two-year period (2006–2007).

Treatment	2006		2007	
	No. of flowers	Fruit set (%)	No. of flowers	Fruit set (%)
Untreated (control)	49	49	71	45
Directly bagged	27	0	24	0
Emasculated and bagged	13	0	32	0
Emasculated and artificially self-pollinated with pollen from the same flower	21	0	26	0
Emasculated and artificially self-pollinated with pollen from another morphologically perfect flower on the same tree	29	0	34	0
Emasculated and artificially cross-pollinated with pollen from a staminate flower	43	16	63	19
Emasculated and netted	15	13	46	15
Emasculated	21	47	28	43

some wind pollination can take place but that it is much less efficient than insect pollination. However, comparing fruit set between untreated (control) flowers and those that were emasculated and artificially cross-pollinated with pollen from male flowers, we found that the latter had lower fruit set (16% vs. 49% in 2006 and 19% vs. 45% in 2007).

DISCUSSION

FUNCTIONAL OR CRYPTIC DIOECY

Barrett (2002) pointed out that it is important to consider the quantitative nature of gender and adopt functional rather than morphological criteria when interpreting plant sexuality. Our study shows that *Nyssa yunnanensis* is characterized by two types of individuals, those producing staminate flowers and those with morphologically perfect flowers that produce sterile, inaperturate pollen. Such apparently androdioecious species are best interpreted as functionally dioecious. At least 78 species in 20 families were recognized by Mayer and Charlesworth (1991) to exhibit functional or cryptic dioecy, and particularly well-known examples include *Solanum appendiculatum* Dunal and *S. asymmetriphyllum* Specht (Levine & Anderson, 1986; Anderson & Symon, 1989).

Why do the morphologically perfect flowers of *Nyssa yunnanensis* bear five to seven stamens that produce inviable pollen? Perhaps to attract and deceive pollinators, as suggested by the fact that the anthers of these flowers are otherwise similar in overall morphology to those of staminate flowers, a

situation also reported in the New Caledonian species *Polyscias pancheri* (Baill.) Harms (Araliaceae) (Schlessman et al., 1990). Real (1981) reported that bees will visit artificial flowers that offer no reward of any kind, and this type of visual deception can be important to the success of some mating systems (Dafni, 1984). Batra (1999) thought the bright yellow anthers of *N. sylvatica* may be attractive to pollinators, and our results show that fruit set is higher in untreated flowers of *N. yunnanensis* than in emasculated flowers, which supports this hypothesis. Furthermore, the anthers of the functionally female flowers may also provide a landing structure for pollinators in species whose flowers have very small petals, as in *N. yunnanensis*. Thus, although the inaperturate pollen in functionally female flowers is not viable, the presence of anthers that produce pollen (albeit sterile) may nevertheless be important for the reproductive success of the species.

Our observations indicate that *Nyssa yunnanensis* is pollinated by generalist visitors such as bees and flies, and that both nectar and pollen appear to serve as rewards for them. Dioecy may be favored in forest species if pollinators are primarily generalists and if there is increased selection pressure for sex separation (Beach & Bawa, 1980). Batra (1999) reported that pollinators were attracted to the small, greenish, and odorless flowers of *N. sylvatica* by the sparkling nectar in addition to the pollen. Our field observations showed that the nectar of *N. yunnanensis* is fragrant, with an applelike smell, and that it serves as a reward for pollinators as well as other floral visitors. Hence, *N. yunnanensis* can be considered primarily a nectar-

rewarding species in which pollination relies on a system of unidirectional exploitation (Dafni, 1984), with pollen serving as a secondary reward.

PHENOLOGY

Bawa and Opler (1975) reported on the phenological patterns of dioecious trees in tropical forests and contrasted them with those of hermaphroditic taxa. A small number of dioecious species flowered during the dry season (which occurs from January to March in Yunnan), whereas the majority flowered at the dry-wet interface (April and May). *Nyssa yunnanensis* fits this pattern well. At the population level, male flowers opened earlier than functionally female flowers but completed flowering almost simultaneously. We suggest that the precocious opening of a small number of male flowers serves to attract a few early pollinators who may serve in turn to guide or attract others by virtue of their memory and learning ability (Cartwright & Collett, 1983), which may facilitate long-distance cross-pollination. Moreover, the 2-whorled androecium and the sequential dehiscence of anthers in male flowers of *N. yunnanensis* may prolong the functional life span of an individual flower and thereby increase the efficiency of out-crossing. It is possible, however, that pollen discounting may also be taking place (Harder & Wilson, 1998) because the sequence of anther maturation and dehiscence is such that the distance between a dehiscing anther and the one that will reach maturity next is maximized, while the length of time during which an anther bears pollen is shortened. This may be an effective way of making the best use of a limited resource for the plant.

In *Nyssa yunnanensis*, the number of flowers in an inflorescence differs between males and females. Anther size also differs strikingly; anthers of the male flowers are ca. 5×3 mm, whereas those of the female flowers are ca. 0.5×0.2 mm (Sun & Zhang, 2007). When differences in the total numbers of male and female flowers within an inflorescence and on an entire plant are also taken into consideration, it is clear that male individuals produce more pollen grains than female plants.

MATING SYSTEM

Because male flowers produce many more pollen grains than functionally female flowers and pollen from the latter is sterile, *Nyssa yunnanensis* can be regarded as xenogamous. Our experiments showed that fruit set was higher in untreated (control) flowers than in flowers subjected to the various treatments in our experimental protocol, especially when female flowers were emasculated and artificially pollinated

using stamens from male trees. This result may be explained by differences in the position of the flowers selected for the treatments. In general, flowers located high on a tree were left as controls and those lower down were treated because they were physically easier to access and manipulate. Because flowers positioned higher on a tree almost certainly received more light, this may have affected fruit set (Cipollini & Stiles, 1991). Parthenocarpic fruit development could, however, be ruled out in *N. yunnanensis* because no fruit was produced by flowers that were emasculated and bagged. Also, flowers that were emasculated and artificially pollinated from male individuals had lower fruit set than untreated flowers, possibly indicating out-crossing depression (see Fischer & Matthies, 1997), although this should be tested further to ensure that no errors occurred during the process of artificial pollination. Lastly, Whitehead (1969) considered that wind pollination is probably uncommon or even absent among tropical forest taxa. Our study suggests, however, that both wind and insect pollination occur in *N. yunnanensis* because fruits were produced in treatments where flowers were emasculated and netted, and fruit set was higher in emasculated flowers that were not netted. This may be related to the fact that adult trees of *N. yunnanensis* are very tall, extending above the denser parts of the forest canopy where wind speed is not significantly reduced.

SEX RATIO WITHIN POPULATION AND AMONG FLOWERS

In dioecious plant species, male and female individuals often show secondary intersexual differences that can be related to the differential constraints and selection pressures imposed on male and female functions (Lloyd & Webb, 1977). Several studies have shown that a 1:1 primary sex ratio often occurs in dioecious species (Fisher, 1930), and deviations from this have been the focus of many theoretical and empirical studies (e.g., Melampy & Howe, 1977; Shea et al., 1993; María & Ramón, 1995; Queenborough et al., 2007). Our results showed a female-biased sex ratio of 0.57:1 in *Nyssa yunnanensis*, adding to only a few previously reported cases in dioecious species (e.g., Melampy & Howe, 1977; Opler & Bawa, 1978; Sassaman, 1991). Opler and Bawa's (1978) study of tropical forest trees indicated that a female-biased sex ratio tends to occur in species whose population densities are high. However, the ratio of the total number of male to female flowers in *N. yunnanensis* shows a different pattern, with a male bias of 2.56:1. This may be explained by the fact that about twice as many flowers are produced per inflorescence on male trees, which also bear more than twice as many inflorescences per branch compared to female trees,

although the branch per tree ratio and the overall sex ratio are both less than 1:1. Differential resource allocation to reproductive function between males and females may translate into differences in trade-offs between vegetative and reproductive activities (María & Ramón, 1995). Males of *N. yunnanensis* produce more flowers than females, indirectly supporting the hypothesis that female plants incur a higher cost of sexual reproduction and that this higher cost is measurable as reduced vegetative growth (male trees bear fewer branches than females) and lower flowering frequency (also see Cipollini & Stiles, 1991).

One of the populations at the Puwen Tropical Forestry Station, the Well population, comprises only functionally female individuals and lacks male individuals altogether, yet no parthenogenesis was detected. Chase et al. (1996) reported that the longest intrapopulation gene-flow distance covered for tropical trees was approximately 350 m. If this is true for *Nyssa yunnanensis* then gene transmission must have taken place between the Stream population and the Well population, which are about 100 m from one another, although we cannot rule out the possibility that some pollen may also be transported from the Dam population located about 2.5 km away.

CONSERVATION IMPLICATIONS

Rarity in plants may be the result of intrinsic or extrinsic (environmental) factors and is mainly related to reproduction (Rabinowitz, 1981; Falk & Holsinger, 1991). Ashton (1977) indicated that apomixes or gene fixation in small populations of self-compatible individuals must be regarded as an evolutionary dead end and would preclude inevitable extinction in a continuously changing biotic environment. However, in our study, despite the very small population sizes observed in *Nyssa yunnanensis*, we found no evidence of apomixes.

Biological diversity is especially high in the humid tropics, but human interference in tropical ecosystems almost inevitably reduces species richness and is recognized as the major cause of the loss of global biodiversity (Turner et al., 1994). The highly restricted populations of *Nyssa yunnanensis* appear to have long suffered from various types of human disturbance such as the building of a dam to supply the water for people around the Puwen Tropical Forest Station and the conversion of large areas of forest to cultivate economically valuable species such as rubber, bananas, and pineapples. Moreover, although the species has been reported as valuable for construction, furniture, decoration, and as an ornamental for landscaping (Wu & Fan, 1977; Song et al., 1989), the local people regard its wood as too soft to be of much use, even as fuel, so

they simply fell the trees when clearing for agriculture. This suggests that the small population size of *N. yunnanensis* seen today may be relictual, i.e., that the 37 trees we studied may be all that remain of a once more widely distributed and abundant species whose range has been severely reduced as a result of human disturbance. If this is the case, the biased sex ratio among the remaining trees may exacerbate and further compound the extrinsic threats faced by *N. yunnanensis*, regardless of whether the observed sex ratio has always been a characteristic of the species or has resulted directly or indirectly from human-caused reduction in population size.

The long-term survival of critically endangered species such as *Nyssa yunnanensis* and hundreds of others in Yunnan Province (Gong et al., 2006) and elsewhere in China (Fu, 1989, 1992), as well as throughout eastern Asia (IUCN, 2008), is intimately dependent on developing and implementing sound in situ conservation policies (supplemented when necessary by ex situ measures such as seed banks and cultivation in botanical gardens) coupled with responsible management of natural resources and promoting improved environmental education and public awareness campaigns. In the case of *N. yunnanensis*, germination and recruitment in the three populations at the Puwen Tropical Forest Station appear to be hampered by an unnaturally dense herbaceous layer that prevents sufficient light from reaching the ground. In an effort to ensure the long-term survival of this rare local endemic, more than 200 individuals are now being grown at Puwen and at the Kunming Institute of Botany, which could be used for a coordinated program involving in situ reintroduction, reinforced local protection, and a simple program to monitor its survival and reproduction, coupled with ex situ efforts to maintain genetic diversity and promote the cultivation of trees in appropriate reserves and botanical gardens. As the present study shows, however, such efforts must be informed by research on the population structure and reproductive biology of individual species, which may also be of great importance and may become even more so as the impacts of projected global and regional climate change begin to alter many complex and often interrelated ecosystem attributes such as flowering and fruiting phenology, pollinator behavior, and microclimate.

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Appendix 1. Pollinators and visitors of *Nyssa yunnanensis*.

Species	Family	Voucher ¹	Observed on		Reward
			Male tree	Female tree	
<i>Nepititsnata adipala</i> Moore	Nymphalidae	Sun2006032806	+	+	nectar
<i>Cethosia bibles bibles</i> (Drury)	Nymphalidae	Sun2006031803	+	+	nectar
<i>Delias hyparete</i> L.	Pieridae	Sun2006031801	+	+	nectar
<i>Ypthima medusa</i> Leech	Satyridae	Sun20060315	+	+	nectar
<i>Lethe</i> Hübner sp.	Satyridae	Sun2006031601	+	+	nectar
<i>Polyhachis</i> Fr. Smith sp.	Formicidae	Sun2006032005	+	+	nectar
<i>Monomorium orientale</i> Mayr	Formicidae	Sun2006031301	+	–	nectar
<i>Monomorium chinense</i> Santschi	Formicidae	Sun2006022901	+	+	nectar
<i>Apis cerana</i> Fabr.	Apidae	Sun2006030604	+	+	nectar, pollen
<i>Monomorium pharaonis</i> (L.)	Formicidae	Sun2006022805	+	+	nectar
<i>Polistes</i> Latreille sp.	Vespidae	Sun2006030601	+	+	nectar, pollen
<i>Myrmosa melanocephala</i> (Fabr.)	Tiphiidae	Sun2006031205	+	–	pollen
<i>Mutilla marginata</i> Baer	Tiphiidae	Sun2006031304	+	+	nectar, pollen
<i>Praestochrysis</i> Linsenmaier sp.	Chrysididae	Sun2006031603	+	–	nectar, pollen
Eurytomidae sp.	Eurytomidae	Sun2006030608	+	–	nectar, pollen
Curculionidae sp.	Curculionidae	Sun2006032801	–	+	nectar
<i>Dinoderus</i> Stephens sp.	Bostrichidae	Sun2006030603	+	–	nectar
<i>Donacia</i> Fabr. sp.	Chrysomelidae	Sun20060305	+	+	nectar
<i>Sphenocorynes</i> sp. Schönherr	Curculionidae	Sun2006031206	+	–	nectar
<i>Gibbium</i> Scopoli sp.	Ptinidae	Sun2006030503	+	–	nectar
<i>Tethina cinerea</i> Loew	Tethinidae	Sun2006031707	+	+	nectar, pollen
<i>Chrysomyia megacephala</i> Fabr.	Calliphoridae	Sun20060307	+	+	nectar, pollen
<i>Iphiaulax imposter</i> (Scopoli)	Braconidae	Sun2006032806	–	+	nectar
Syrphidae sp. (f)	Syrphidae	Sun2006031701	+	+	nectar
Syrphidae sp. (m)	Syrphidae	Sun2006031701	+	+	nectar
<i>Psorophory</i> sp1	Gulicidae	Sun2006030701	+	–	nectar
<i>Psorophory</i> sp2	Gulicidae	Sun2006030706	+	–	nectar
<i>Psorophory</i> sp3	Gulicidae	Sun2006030601	+	–	nectar
<i>Tipula</i> L. sp.	Tipulidae	Sun2006031901	+	–	nectar
<i>Fannia</i> Robineau-Desvoidy sp.	Fanniidae	Sun2006030702	+	+	nectar, pollen

Appendix 1. Continued.

Species	Family	Voucher ¹	Observed on		Reward
			Male tree	Female tree	
<i>Parasarcophaga</i> Johnston & Tiegs sp.	Sarcophagidae	Sun2006031204	+	+	nectar
<i>Euxesta</i> Loew. sp.	Ulidiidae	Sun2006030610	+	–	nectar
<i>Cobolidia fuscipes</i> (Meigen)	Scatopsidae	Sun2006030602	+	+	nectar
<i>Stratiomyia</i> sp1	Stratiomyidae	Sun2006030504	+	+	nectar
<i>Stratiomyia</i> sp2	Stratiomyidae	Sun20060301	+	–	nectar
<i>Blattella</i> Caudell sp.	Blattaria	Sun2006032004	+	–	nectar

+, visiting flower; –, not visiting flower.

¹ Vouchers deposited at the Herbarium of the Kunming Institute of Botany (KUN).