American Journal of Botany 92(3): 517–524. 2005.

PREDICTING MATING PATTERNS FROM POLLINATION SYNDROMES: THE CASE OF "SAPROMYIOPHILY" IN *TACCA CHANTRIERI* (TACCACEAE)[1]

LING ZHANG,[2] SPENCER C. H. BARRETT,[3] JIANG-YUN GAO,[2] JIN CHEN,[2] W. W. COLE,[3] YONG LIU,[2] ZHI-LIN BAI,[2] AND QING-JUN LI[2,4]

[2]Xishuangbanna Tropical Botanical Garden, The Chinese Academy of Sciences, Yunnan 666303, China; and [3]Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2

Tacca, a genus of tropical herbs, possesses near black flowers, conspicuous involucral bracts and whisker-like filiform bracteoles. These features have been assumed to function as a "deceit syndrome" in which reproductive structures resemble decaying organic material attracting flies that facilitate cross-pollination (sapromyiophily). We investigated pollination and mating in *Tacca chantrieri* populations from SW China to evaluate this assumption. Contrary to this expectation, populations were highly selfing. Pollinator visitation was infrequent and bagged flowers set abundant seed. Pollen loads on stigmas indicated autonomous self-pollination, some of which occurred prior to flower opening. The seed set of inflorescences with bracts and bracteoles removed was not significantly different from unmanipulated inflorescences, suggesting that these structures play a limited role in pollinator attraction, at least at our study sites. Pollen : ovule ratios averaged 49, a value expected in a highly selfing species. Selfing rates estimated in four populations using allozyme markers averaged 0.86 (range 0.76–0.94), thus corroborating this inference. Our results indicate that despite considerable investment in extravagant display, populations of *T. chantrieri* are highly selfing. We propose several hypotheses to resolve this paradox and argue that future studies of pollination syndromes would benefit by investigation of both pollination and mating biology.

Key words: autonomous self-pollination; deceit pollination; extravagant display; mating system; prior selfing; reproductive assurance; sapromyiophily.

Floral displays in angiosperms function by attracting animal pollinators to plants, thus promoting pollen dispersal and cross-pollination. In many species, particular groups of animal pollinators have shaped floral evolution resulting in the recognition of distinct pollination syndromes (Fægri and van der Pijl, 1971; Proctor et al., 1996; Fenster et al., 2004). The genetic benefits that result from cross- vs. self-fertilization have been widely recognized since Darwin's pioneering studies on this topic (Darwin, 1876; Charlesworth and Charlesworth, 1987a). Although most displays involve elaborate floral designs or the aggregation of flowers into showy inflorescences, in some families nonfloral structures are the principal organs of pollinator attraction. For example, in small-flowered species of Araceae, Bromeliaceae, Cornaceae, Euphorbiaceae, and Rubiaceae, showy petaloid bracts probably function in pollinator attraction by enhancing visual display (Weberling, 1992). Investment in attractive structures represents an allocation cost that animal-pollinated plants pay to secure the fitness advantages that accrue from cross-fertilization (Charlesworth and Charlesworth, 1987b). In contrast, for species in which predominant selfing is the primary mating strategy, investment in attractive structures is superfluous, and resources are instead redirected to alternative structures or activities (Charnov, 1982; Lloyd, 1987). These considerations lead to the expectation that if a species has high investment in extravagant displays, it should be largely outcrossing.

Tacca is comprised of ~10 species of acaulescent forest understory herbs and is the only genus of the monocot family Taccaceae (but see Ding and Larsen, 2000). *Tacca* is primarily Palaeotropical in distribution, centered in Indomalesia, China, and Southeast Asia (Drenth, 1972). *Tacca* species have become increasingly popular in the horticultural trade because of their bizarre reproductive morphology involving cymose umbellate inflorescences subtended by large conspicuous bracts, and many long, whisker-like filiform bracteoles. *Tacca* flowers are dark purple, brown, or near black in color and are actinomorphic, hermaphroditic with six stamens. Hereafter, we refer to the entire inflorescence with associated bracts and bracteoles as "floral display," recognizing that these structures include nonfloral parts.

Despite the unusual reproductive morphology of *Tacca*, there have been no detailed investigations of the pollination and mating biology of species in the genus. Drenth (1972) and Saw (1993) assumed that *Tacca* species have the sapromyiophilous syndrome, in which the color and odor of flowers mimic rotting organic material and cross-pollination is mediated by flies. Stevenson (2004) also suggested that the dark-colored flowers and large bracts were likely to be associated with fly pollination (and see Endress, 1995). Dark floral colors, the presence of long filiform appendages or bracts, floral traps, the absence of nectar, and a decaying odor are common features of the sapromyiophilous syndrome in other families of flowering plants (e.g., Asclepiadaceae, Aristolochiaceae, Araceae, Orchidaceae; reviewed in Fægri and van der Pijl, 1971; Proctor et al., 1996). These traits are all reported in species of *Tacca* and have been interpreted in the context of the sapromyiophilous syndrome (Drenth, 1972; Saw, 1993).

[1] Manuscript received 3 May 2004; revision accepted 25 November 2004.

We thank Drs. Amots Dafni, W. John Kress, Jeff Ollerton, and Shuang-Quan Huang for constructive discussions and comments on the manuscript; Mr. Neil White for assistance with allozyme analysis; Mr. Xiao-Dong Yang and Pan-Yu Ren for some art work on photographs; Dr. L. G. Saw for providing his reference on *Tacca*, and Dr. S.-Q. Huang for pollen and insect scanning electron microscope observations. This work was funded by NSFC grant 30225007 to Q.-J. Li, the Yunnan Provincial Natural Science Foundation grant 2003C0024Q, the Ten-Thousand-Species Project grant of XTBG to L. Zhang, and a Discovery Grant from NSERC Canada to S. C. H. Barrett.

[4] Author for correspondence (e-mail: qjlixtbg@bn.yn.cninfo.net).

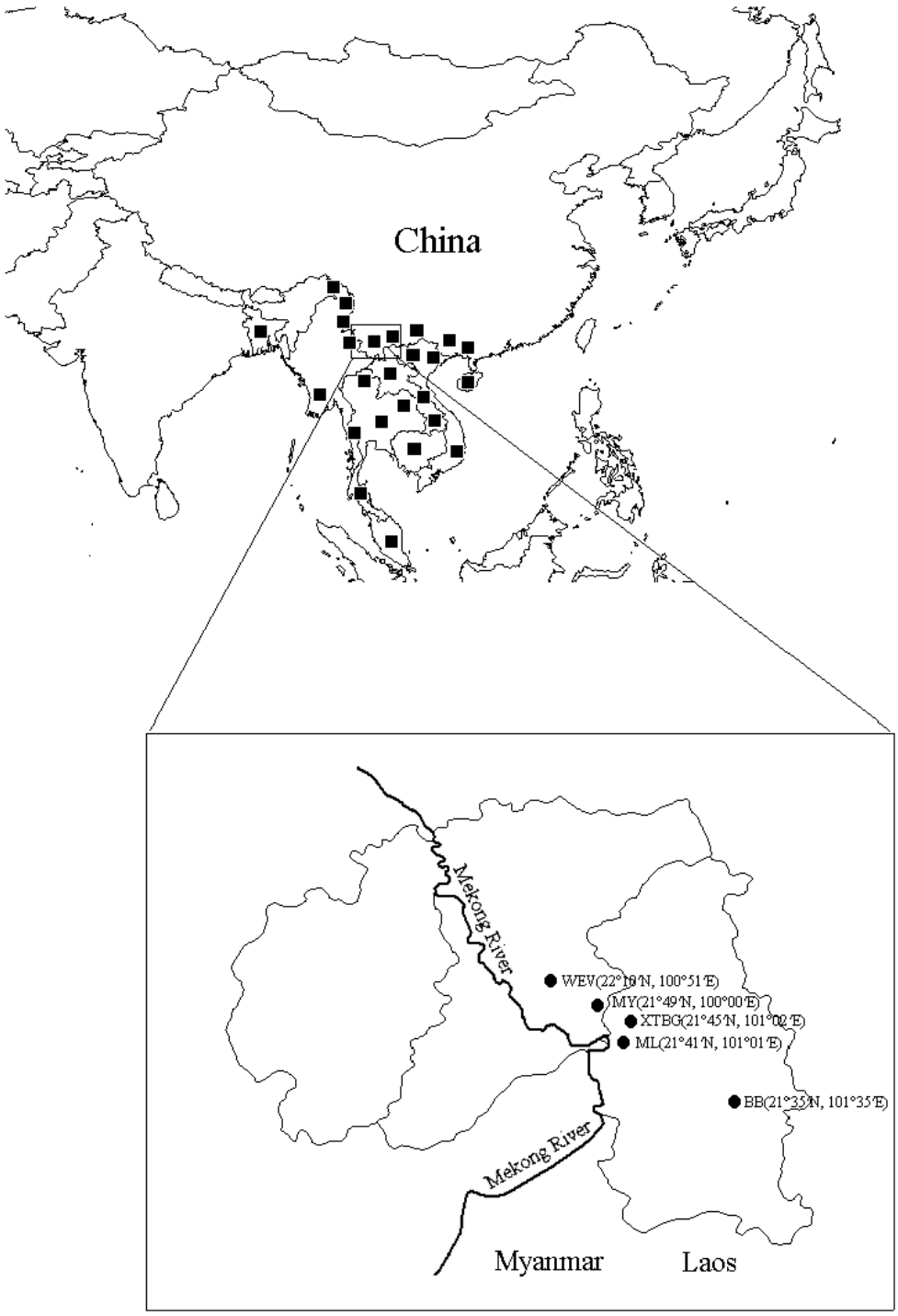


Fig. 1. The distribution of *Tacca chantrieri* (from the website of the Missouri Botanical Garden, www.mobot.org) and the locations of populations investigated in this study in South Yunnan province, China.

Here we investigate the floral biology of *T. chantrieri* André, an endangered species (Fu and Jin, 1992) that occurs in tropical regions of SE Asia including Thailand, Malaysia, and southern China, particularly Yunnan Province (Fig. 1). This species has four large, dark-purple bracts and long bracteoles, giving the inflorescence a striking appearance in the understory of the tropical forests that the species inhabits (Fig. 2a, b). In areas where *T. chantrieri* occurs, local inhabitants liken the appearance of the species to a flying bat, a sinister face, or a mean tiger with whiskers. Indeed, several of the vernacular names used for *T. chantrieri* (''bat plant,'' ''tiger's whisker,'' or ''devil flower'') reflect these images. The main objective of our study was to establish whether or not the unusual features of flower and inflorescence morphology in *T. chantrieri* are part of a syndrome associated with sapromyiophily, as proposed in the literature for *Tacca* species in general.



Fig. 2. Reproductive biology of *Tacca chantrieri*. (a) The forest habitat. (b) The inflorescence display with prominent, dark purple bracts and whisker-like bracteoles. (c) A cross-section of the flower illustrating the close proximity of the stigma and anthers. (d) The infructescence with relatively high fruit set. (e) The nectarless flower with a small pollen-collecting stingless bee (*Trigona* sp.). (f) Pollen grains of *T. chantrieri* adhering to the leg of a stingless bee.

We begin by describing the floral biology of *T. chantrieri*, focusing on traits associated with pollination and mating. We then address the following specific questions: (1) How is pollination achieved and what is the functional significance of the extravagant display? (2) Are flowers susceptible to self-pollination and to what extent are insect pollinators necessary for seed production? (3) Given the floral biology of *T. chantrieri*, what are the realized patterns of mating in natural populations? Following the presentation of our results, we discuss the relations between floral traits and mating and propose several explanations for our unexpected discovery that populations of *T. chantrieri* are predominantly self-fertilizing.

MATERIALS AND METHODS

Study species and sites—*Tacca chantrieri* inhabits moist and shaded understory habitats in tropical forests of SE Asia (Fig. 1). Plants are 50–100 cm tall with tubers or creeping rhizomes and alternate, elliptic, entire leaves. In Yunnan Province, SW China, the region in which our studies were conducted, the species flowers from April to July. We made field observations and experiments at two locations over a 3-yr period (2001–2003) in seasonal forests. The first site was at Wild Elephant Valley (WEV) in Mengyang Nature Reserve, (22°10′ N, 100°51′ E; 760 m a.s.l.) and is dominated by *Pometia tomentosa* (Sapindaceae). The second site was in a protected area of Xishuangbanna Tropical Botanical Garden (XTBG; 21°45′ N, 101°02′ E; 580 m a.s.l.) that is dominated by *Tetrameles nudiflora* (Datiscaceae) and *Horsfieldia*

tetratepala (Myristicaceae). We also transplanted individuals from natural populations around XTBG to a shady location at XTBG to observe flowering phenology in detail. Finally, we estimated mating patterns (see later) in four populations occurring at the following locations: Bubeng dipterocarp forest (*Shorea wangtianshunea*, Dipterocarpaceae) in Mengla County (BB); Wild Elephant Valley in Jinghong County (WEV); Menglun nature reserve in Mengla County (ML); protected forest close to the Mengyang-Menglun highway (MY), 43 km from Mengyang in Jinghong County (Fig. 1).

Floral biology—We made preliminary phenological observations in natural populations and monitored in detail flowering and fruiting on transplants obtained from the Menglun population during 2001–2003. We randomly selected 30 inflorescences, one per plant, and recorded the total number of flowers, the duration of flowering, the number of filiform bracteoles, and for one flower per inflorescence, we estimated the number of pollen grains and ovules. We used a haemocytometer to estimate pollen production per flower following methods in Dafni (1992). We used data from pollen and ovule number to calculate the mean pollen : ovule ratio (P/O) of flowers. To investigate floral behavior during anthesis, we monitored three inflorescences every 2 h during the day for 2 d by recording the timing of anther dehiscence and flower position (measured as the angle between the flower pedicel and the inflorescence scape). To determine if changes in flower position promoted self-pollination, we counted pollen grains on the stigma at three time periods: (1) 0630 hours, 4 h before flower opening; (2) 1030 hours, right after flower opening; (3) 2230 hours, 12 h after flower opening at which time flowers were pendant.

Flower visitors and pollination—We observed flower visitors to *T. chantrieri* in 2002 and 2003 at WEV and XTBG for a total of 90 and 48 h, respectively, during favorable weather. Observations were made continuously from 0900 hours when flowers began opening to around 1900 hours when they became pendant. We recorded the total number of flower visitors per individual and the number of flowers visited. We assigned visitors to one of two groups (visitors and pollinators), based on their behavior and likelihood of mediating pollination. Visitors were insects observed on inflorescences, whereas pollinators were insects that consistently contacted both anthers and stigmas and had pollen grains deposited on their bodies. We photographed all types of flower visitors, and voucher specimens of insects are preserved in the insect collections of XTBG.

Field manipulative experiment—To investigate if the showy bracts and long filiform bracteoles function in pollinator attraction, we conducted a manipulative field experiment at WEV in which plants were exposed to natural pollination. We set up three treatments with 30 individuals per treatment using one randomly chosen inflorescence per plant. The three treatments were: (1) an unmanipulated control, (2) the removal of bracts and bracteoles, and (3) staked inflorescence. For treatment 3, the focal inflorescence was prevented from moving in the wind by securing the inflorescence axis (scape) to a wooden stick. This reduced the motility of the long filiform bracteoles, which, as proposed by Fægri and van der Pijl (1971), may serve in pollinator attraction. Four months later when fruits were mature, we counted the fruit set and seed set (seed : ovule ratio) of inflorescences.

Controlled pollination experiment—We performed two pollination treatments on a single inflorescence of 20–26 randomly selected individuals of *T. chantrieri* at the WEV and XTBG populations to examine the capacity for autonomous self-pollination and therefore the importance of insect visitors to pollination. The treatments were: (1) open-pollinated controls and (2) autonomous self-pollination. For treatment (2), we bagged inflorescences on each individual prior to anthesis to prevent access by pollinators. We also performed hand cross-pollinations using pollen from plants up to 10 m away on 35 flowers randomly selected from 10 inflorescences. Two days prior to anthesis, the flowers were emasculated to prevent prior self-pollination. For this treatment, not all flowers within an inflorescence were cross-pollinated because of the technical difficulties associated with bud emasculation in this species. We analyzed the fruit and seed set data for the open-pollinated and bagged flowers using a two-way ANOVA (JMP ver. 4.0.4, SAS Institute, 2002) with both population and pollination treatment as fixed effects. Fruit set data were arcsine transformed prior to analysis.

Mating patterns—We estimated mating patterns in four populations of *T. chantieri* using 16 open-pollinated families sampled at random from each population. We estimated selfing rates in each population based on polymorphism at three allozyme loci resolved by starch gel electrophoresis. We ground seeds in a sodium phosphate extraction buffer (20 mg diethyl-dithio-carbamic acid, 10 mg EDTA, 25 mg BSA and one drop of Tween 80 dissolved in 20 ml of 0.05 M sodium phosphate pH 7.0). The extracts were adsorbed onto chromatography-paper wicks (Whatman's 17 wicks, Maidstone, UK), and placed directly onto 12% starch gels (1 : 2, hydrolyzed potato starch, S-5651 [Sigma, Missouri, USA] and CAT 32823 [USB Corporation Ohio, USA]). We initially screened for electrophoretic variation in enzymes on two buffer systems: aspartate amino transferase (AAT), alcohol dehydrogenase (ADH), glutamate dehydrogenase (GDH), cytosol aminopeptidase (LAP), and triose phosphate isomerase (TPI), on lithium-borate (pH 8.3); and aconitase (ACO), acid phosphotase (ACP), isocitric dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PDH), malate dehydrogenase (MDH), phosphoglucose dehydrogenase (6PGD), phosphoglucose isomerase (PGI), shikimic dehydrogenase (SkDH) on histidine-citrate (pH 6.5). Gels were stained for enzyme activity following recipes in Wendel and Weeden (1991). We used variation at three polymorphic loci (MDH, GDH, G6PDH) to estimate multi-locus selfing rates ($s_M = 1 - t_M$) and parental inbreeding coefficients (F) using the computer program MLTR version 2.2 (Ritland, 2002). The remaining loci had limited or no variation or could not be reliably scored. We used the Newton Raphson iteration option to find the maximum-likelihood estimate of the selfing rate and parental inbreeding coefficient. We derived the standard errors of selfing rate estimates from the standard deviation of 1000 bootstrap values, using the seed family as the unit of resampling. We constrained pollen and ovule allele frequencies to be equal to each other to maximize the accuracy of the mating system estimates.

RESULTS

Floral biology—*Flowering phenology*—Observations of flowering at WEV indicate that flowers of *T. chantrieri* begin anthesis around 1000 to 1100 hours and last for 2 d. Inflorescences generally flower for 6–14 d (10.1 ± 0.3, mean ± SE, $N = 50$) and produce 1–4 (1.7 ± 0.78, $N = 100$) flowers each day. Flowering occurs mainly from early April to mid-May, with sporadic inflorescences being produced until early July.

Floral characteristics—Plants at WEV produced 7–20 (13.2 ± 0.5, $N = 50$) flowers per inflorescence. Values for percentage fruit set (fruit : flower ratio) per inflorescence and percentage seed set (seed : ovule ratio) per fruit at WEV were 69.6 ± 19.9 (30.8–100, $N = 20$) and 56.0 ± 14.8 (23.2–75.0, $N = 20$), respectively. Corresponding values for XTBG were 10–27 (16.0 ± 0.6, $N = 50$) flowers per inflorescence, and 62.85 ± 15.2 (31.6–86.7, $N = 25$) fruit set and 49.2 ± 8.3 (33.1–64.4, $N = 25$) seed set, respectively (Table 1). The two populations differed significantly in the number of flowers per inflorescence ($t = 3.81$, $P = 0.0001$, df = 98) and seed set ($t = 1.98$, $P = 0.03$, df = 43). Flowers of *T. chantrieri* do not produce nectar or any odor that we were able to detect. The number of pollen grains and ovules produced per flower in plants from XTBG population was 14 021 ± 510 (range 6761–18 903, $N = 30$) and 285 ± 7.1 (range 215–474, $N = 30$), respectively. Hence the P : O (pollen : ovule) ratio of *T. chantrieri* is 49 (Table1).

Floral behavior during anthesis—Prior to anthesis, the cymose umbellate inflorescences of *T. chantrieri* are surrounded

TABLE 1. Floral characters of *Tacca chantrieri* based on samples from the XTBG population.

	No. of flowers/ inflorescence	Duration of an inflorescence (d)	No. of bracteoles	Pollen grains	Ovules	P/O ratio
Mean ± SE	13.2 ± 0.5	10.1 ± 0.3	21.4 ± 0.6	14021 ± 510	285 ± 7	49
Range	7–20	6–14	17–26	6761–18903	215–474	—
N	50	50	27	30	30	30

Note: P/O = pollen : ovule ratio.

by four involucral bracts (two large and two small, see Fig. 2b), the bracts separate at the beginning of flowering. Anthesis commences at the center of the inflorescence with one to four flowers (usually one or two) in anthesis each day. Flower buds and 1-d-old flowers are held erect but bend downwards during the evening of the first day of anthesis. Significantly, anthers dehisce several hours before flowers have opened, and because of the close proximity of anthers and stigmas, pollen grains are deposited on the stigmatic surface in large numbers. As anthesis proceeds and flowers become pendant, additional pollen grains accumulate on the stigma through autonomous intrafloral self-pollination (Fig. 3).

Pollination ecology—Few insects were observed on flowers of *T. chantrieri* at XTBG and WEV despite more than 138 h of observation during the flowering seasons of 2001 and 2003. At XTBG, we observed occasional crickets and ants on flowers; neither of these visitors were effective as pollinators because sex organs were not contacted. At WEV, we observed occasional visits to plants by a few ants, spiders, and flies, but these visits were also not effective in pollination. The only genuine pollinators that we observed in either population were stingless bees (*Trigona* sp.) at WEV. These bees were observed collecting pollen and contacting both the stigma and anthers (Fig. 2e). We confirmed with SEM images that pollen grains are deposited on the bodies of *Trigona* (Fig. 2f). Overall visitation rates of *Trigona* at WEV were 0.93 ± 0.22 (range 0.43–1.53, $N = 6$) visits/flower/h. and individual bees did not discriminate between first day (upright) and second day (pendant) flowers.

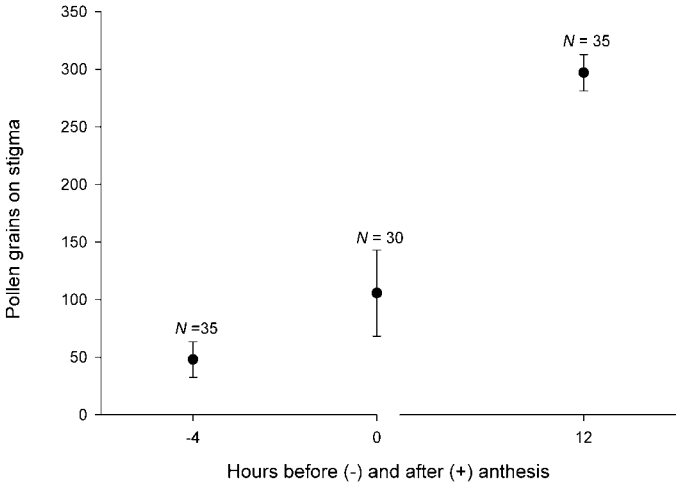


Fig. 3. The number of pollen grains deposited autonomously on the stigma surface of *Tacca chantrieri* flowers at three time intervals in relation to anthesis.

The function of bracts and bracteoles—In our field experiment, the percentage fruit set and seed set did not differ significantly among the three treatments (fruit set: control, 67.8 ± 3.69 [mean ± SE, $N = 23$]; bracts removed, 65.2 ± 3.74 [$N = 23$]; staked, 72.7 ± 3.52 [$N = 25$]; seed set: control, 45.8 ± 3.29; bracts removed, 44.6 ± 2.36; staked, 47.9 ± 2.77; $P > 0.1$ for all comparisons). The inflorescence number for each treatment was initially 30. However, when fruits were mature 4 mo later, samples sizes were reduced because of damage by elephant trampling.

Autonomous self-pollination—There was no significant difference in the fruit set of open-pollinated (control) and bagged inflorescences of *T. chantrieri* at XTBG and WEV. However, overall fruit set was ~10% lower at XTBG (two-way ANOVA on arcsine transformed fruit set, $F_{3,92} = 3.77$, $P = 0.013$, population, $F_{1,92} = 10.012$, $P = 0.002$, pollination treatment, $F_{1,92} = 0.10$, $P = 0.748$ NS and population × pollination treatment interaction, $F_{1,92} = 0.57$, $P = 0.453$ NS). Mean percentage fruit set of bagged inflorescences was 74.1 ± 3.2 ($N = 26$) at WEV and 61.2 ± 3.2 ($N = 25$) at XTBG. Fruit set of the control treatment was 69.6 ± 3.6 ($N = 20$) at WEV and 62.8 ± 3.3 ($N = 25$) at XTBG.

Similar analysis of seed : ovule ratio indicated no evidence of a population effect but a significant effect of pollination treatment and population × treatment interaction (two-way ANOVA on seed set, $F_{3,92} = 2.69$, $P = 0.05$, population, $F_{1,92} = 0.40$, $P = 0.528$ NS, pollination treatment $F_{1,92} = 4.43$, $P = 0.038$ and population × pollination treatment interaction $F_{1,92} = 3.90$, $P = 0.05$). Further ANOVA of percentage seed set data by population indicated no significant difference at XTBG ($F_{1,48} = 0.018$, $P = 0.895$ NS, bagged seed set = 48.8 ± 1.83, $N = 25$, control = 49.1 ± 1.83, $N = 25$) but significantly more seed set in the control treatment at WEV ($F_{1,44} = 5.16$, $P = 0.028$, bagged seed set = 45.3 ± 3.12, $N = 26$, control = 56.04 ± 3.57, $N = 20$).

Percentage fruit set in hand cross-pollinations was 25.7, with the percentage seed set of fruits 42.3 ± 7.3. The low value for fruit set in comparison with open-pollinated and bagged flowers may reflect mechanical damage associated with emasculation or low viability of cross pollen. When flowers were emasculated just before opening, they produced 42.3% ± 4.1 fruit set and 28.4% ± 3.24 seed set. This confirms that autonomous self-pollination prior to flower opening produces a significant quantity of seed.

Mating patterns—Among the four populations of *T. chantrieri* examined in this study (Table 2), estimates of the population-level maternal selfing rate (s_m) based on three polymorphic allozyme loci averaged 0.86 (range 0.76–0.94). This indicates that a high proportion of the seed produced in each population results from self-fertilization. As expected, based on these mating patterns, measurements of the parental in-

TABLE 2. Parental inbreeding coefficient and multilocus selfing rates ($s_m \pm$ SE) in four populations of *Tacca chantrieri* from Yunnan Province, SW China.

Population code[a]	Population size	Sample size *N* family/progeny	Number of loci	*F* of parents	Multilocus selfing s_m
BB	~180	16/112	3	0.394 (0.084)	0.859 (0.066)
WEV	~150	16/122	3	0.296 (0.095)	0.941 (0.032)
ML	~70	16/126	3	0.565 (0.057)	0.755 (0.053)
MY	~60	16/117	3	0.754 (0.040)	0.896 (0.039)

[a] BB = Bubeng, WEV = Wild Elephant Valley, ML = Menglun, MY = Mengyang.

breeding coefficients (*F*) indicate that the parental generation is significantly inbred (Table 2).

DISCUSSION

Pollination syndromes are suites of convergent traits that have evolved independently in unrelated plant lineages functioning to attract particular groups of animal pollinators (e.g., bird, bat, fly, hawkmoth) (Fægri and van der Pijl, 1971; Proctor et al., 1996). Although the possession of a particular syndrome rarely restricts visitation to a single animal group (Baker, 1963; Waser et al., 1996; Johnson and Steiner, 2000), there is general agreement that pollination syndromes have been shaped by past coevolution between plants and their most effective pollinators (Stebbins, 1970; Fenster et al., 2004; but see Aigner, 2001). Regardless of the degree of pollinator specialization involved, a rarely tested but fundamental assumption is that the ultimate function of pollination syndromes is to promote cross-pollination because of the fitness benefits obtained from outcrossing. The main finding of our study points to the need to confirm this assumption and warns against inferring patterns of mating from floral morphology alone. Despite possessing a suite of traits usually associated with sapromyiophily, our investigations of *T. chantrieri* failed to reveal deceit pollination mediated by flies. Instead, our results indicate that populations of this species are predominantly selfing and that flowers have several traits that promote autonomous self-pollination. We propose several explanations that help to resolve this paradox.

With little doubt, as described by Drenth (1972) and Saw (1993), species of *Tacca*, including *T. chantrieri*, possess a suite of traits normally associated with sapromyiophily. These include dark flowers and bracts, motile filiform appendages, a trap mechanism involving the stamens and stigma acting as a hood and the absence of nectar. A fetid odor occurs in many sapromyiophilous species (Faegri and van der Pijl, 1971; Proctor et al., 1996). Saw (1993) reported a musky smell in *T. integrifolia*, but we were unable to detect any odor associated with the flowers or bracts of *T. chantrieri*. Perhaps *T. chantrieri* produces compounds attractive to flies but not perceptible by humans, as is the case in the fly-pollinated *Asarum* (Proctor et al., 1996). Despite the possession of traits commonly associated with fly pollination by deceit, we recorded no flies acting as pollinators of *T. chantrieri*. The only pollinator we observed was *Trigona*, a ubiquitous pollen-collecting tropical bee that visits many species opportunistically (Roubik, 1989) and was seen visiting flowers of *T. chantrieri* at one of our field sites. Our failure to observe fly pollination in *T. chantrieri* is unlikely to be the result of a rarity of flies in the vicinity of the populations we investigated. Indeed, flies are often considered the most dependable of animal pollinators, at least in terms of abundance (Larson et al., 2001). Destabilization of mutualisms can occur if pollination systems are highly specialized and pollinators are lost from a region (Bond, 1994). This seems doubtful in the case of *T. chantrieri*, as extreme specialization would be unlikely in a species in which the only apparent floral reward (pollen) is easily accessible to most insect visitors.

Our studies of the floral biology and mating system of *T. chantrieri* indicate that most seeds produced in populations result from self-fertilization. The low pollen : ovule ratio (49) of *T. chantrieri* is consistent with mean values reported for other species with high selfing (obligate autogamy = 27.7, facultative autogamy = 168.5; Cruden, 1977). Selfing in *T. chantrieri* is promoted by several floral mechanisms that result in autonomous intrafloral self-pollination. These include the close proximity of anthers and stigmas within flowers, anther dehiscence prior to flower opening, and additional self-pollination when flower stalks bend downwards on the second day of anthesis (delayed self-pollination). Pollinator-facilitated, intrafloral and interfloral (geitonogamous) selfing may also contribute to high selfing rates in populations visited by pollinators. However, our finding of similar levels of seed set in bagged and open-pollinated flowers at the XTBG population (although not at WEV) implies that most selfing probably arises by autonomous means. If this is true, why does *T. chantrieri* maintain a suite of floral traits associated with sapromyiophily? It is unlikely that the syndrome is maintained simply to attract flies for the purpose of facilitated intra- and interfloral selfing. We consider next several hypotheses that may explain the association between the sapromyiophilous syndrome and high selfing rates in *T. chantrieri*.

Spatial and temporal variation in mating patterns?—Our study of mating patterns in *T. chantrieri* was conducted in four populations during a single flowering season. The populations were located in a relatively restricted portion of the geographical range of *T. chantrieri* (Fig. 1), and it is possible that in other parts of the range floral traits associated with sapromyiophily function more effectively in promoting fly-mediated cross-pollination. Geographical variation in mating patterns is well known in other species of flowering plants, and in some cases, high selfing is associated with infrequent pollinator service (reviewed in Lloyd, 1980; Barrett et al., 2001). However, in species in which this occurs, reduced investment in both floral and inflorescence display is usually associated with predominant selfing (Ornduff, 1969; Charnov, 1982; Schoen, 1982; Lloyd, 1987; Morgan and Barrett, 1989). Apparently, this has not occurred in *T. chantrieri* despite mechanisms for autonomous selfing and the low pollen : ovule ratio of the species.

It is also possible that the mating patterns in the populations of *T. chantrieri* we examined fluctuate from year to year and

that increased outcrossing is limited to years with high population densities of pollinators. Such temporal variability in selfing rates has been reported in other self-compatible animal-pollinated species (Harding and Barnes, 1977; Barrett et al., 1993). With spatial and temporal variation in pollinator service, even low levels of fly-mediated outcrossing may be sufficient to maintain the sapromyiophilous syndrome, if the fitness benefits of outcrossed progeny are sufficient to outweigh the costs of selfing and investment in display. Unfortunately, a rigorous cost-benefit analysis of the balance between selfing and outcrossing in plants has yet to be undertaken (see Eckert and Herlihy, 2005).

Sapromyiophily as a relict syndrome with no current utility?—The association between high selfing rates and the sapromyiophilous syndrome in *T. chantrieri* may occur because the syndrome is a relict condition with no current functional significance. In some contemporary populations, the syndrome could be selectively neutral, and developmental constraints may have prevented significant evolutionary modifications. For example, the loss of bracts, and to a lesser extent bracteoles, would involve significant re-modeling of plant phenotype and the persistence of these traits may reflect structural constraints to such fundamental changes in morphology. According to this hypothesis, the sapromyiophilous syndrome was adaptive in the ancestors of *T. chantrieri*. Indeed, the syndrome could function in other populations of *T. chantrieri* elsewhere in its range. However, under current ecological conditions in southwest China, the syndrome may have little utility, perhaps because of scarce pollinator service.

The moist forest habitats of *T. chantrieri* are characterized by deep shade, and despite the general abundance of flies in tropical regions, these particular environments may not favor predictable pollinator service. In a consistently pollinator-limited environment, floral adaptations promoting autonomous selfing could have been selected, thus explaining several features of the floral biology of the populations we investigated. The autonomous selfing mechanisms and low pollen : ovule ratio of *T. chantrieri* are certainly consistent with this scenario; however, the sub-maximal fruit set that we recorded in open-pollinated and bagged flowers are not expected in populations with a long history of selfing. In most autogamous plants, fruit to flower ratios are close to 1.0, suggesting that the reproductive economy of the *T. chantrieri* populations that we investigated may not be fully adjusted to high levels of selfing.

Our manipulative field experiment failed to provide evidence for the function of bracts and bracteoles in pollinator attraction. The female fertility of the two experimental treatments was not significantly different from the control treatment. Several deficiencies in our experiment may have prevented us from detecting significant treatment effects. For example, we did not attempt to measure visitation rates because pollinators were so infrequent at the site. Therefore, we have no information on whether our manipulations influenced pollinators in any way. Also, we only compared maternal reproductive success (fruit and seed set) in the three treatments and not male fitness resulting from pollen dispersal between plants. Finally, our manipulations were not conducted at the whole plant level, and our inflorescence level modifications may have been insufficient to illicit differential responses. Nevertheless, despite these shortcomings and given our findings concerning the mating system, it seems probable that most of the seed produced by plants in the experiment (including the control) resulted from self-pollination rather than pollinator-mediated cross-pollination. Elsewhere, Lamborn and Ollerton (2000) used floral manipulations to investigate the function of the dark central floret in inflorescences of *Daucus carota* (Apiaceae). Their experiments provided no evidence for an obvious adaptive role, and they proposed that this structure may be selectively neutral persisting after its original function was lost.

Alternate adaptive functions for traits?—During flowering, the most conspicuous features of display in *T. chantrieri* are the large, purple involucral bracts and long, filiform bracteoles (Fig. 2a, b). Previous workers have assumed that these structures function to attract flies to plants in the shaded understory of tropical forests. Given their leaflike structure and size, the large involucral bracts in *T. chantrieri* could also be a significant source of photosynthetic carbon for developing fruits. However, the vertical orientation of bracts is unlikely to be an optimal orientation for light capture in shady environments, thus casting doubt on this explanation. An alternative possibility is that the bracts and bracteoles function in seed dispersal and serve to attract dispersal agents to plants. *Tacca* species produce fleshy fruits that are assumed to be animal dispersed by birds and small rodents (Saw, 1993). However, a role in dispersal seems unlikely because the bracts and bracteoles wither and senesce before fruits are mature. Therefore, it is hard to imagine how the bracts and bracteoles of *T. chantrieri* might be important in the dispersal of seeds.

Conclusions—We have provided several hypotheses that might explain the association between high selfing and the sapromyiophilous syndrome in populations of *T. chantrieri* in SW China. Further investigations of pollination and mating in *T. chantrieri* are clearly desirable to assess whether low pollinator visitation and high selfing rates are a common feature in this species, and in other *Tacca* species with similar reproductive traits. In addition, our study raises several general questions concerning pollination and mating in animal-pollinated plants. Despite the functional link between these critical stages of sexual reproduction, few studies of pollination syndromes have simultaneously investigated both processes. As a result, we have remarkably little direct evidence on the effectiveness of different pollinator groups in promoting cross-fertilization, or whether some syndromes are more susceptible to self-pollination than others. For example, how effective are flies in promoting outcrossing in other sapromyiophilous groups? It is possible that our results for *T. chantrieri* may not be atypical, especially in groups without effective anti-selfing mechanisms such as self-incompatibility or dioecy. Future studies in pollination biology might consider the measurement of mating parameters to determine if the considerable investment that occurs in display traits is indeed repaid through the benefits of outcrossing.

LITERATURE CITED

AIGNER, P. A. 2001. Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? *Oikos* 95: 177–184.

BAKER, H. G. 1963. Evolutionary mechanisms in pollination biology. *Science* 139: 877–883.

BARRETT, S. C. H., B. C. HUSBAND, AND W. W. COLE. 1993. Variation in outcrossing rates in *Eichhornia paniculata*: temporal changes in populations of contrasting morph structure. *Plant Species Biology* 8: 141–148.

BARRETT, S. C. H., M. E. DORKEN, AND A. C. CASE. 2001. A geographical

context for the evolution of plant reproductive systems. *In* J. Silvertown and J. Antonovics [eds.], Integrating ecological and evolutionary processes in a spatial context, 341–363. Blackwell, Oxford, UK.

BOND, W. J. 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London, Series B* 344: 83–90.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987a. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987b. The effect of investment in attractive structures on allocation to male and female functions in plants. *Evolution* 41: 948–968.

CHARNOV, E. L. 1982. The theory of sex allocation. Princeton University Press, Princeton, New Jersey, USA.

CRUDEN, R. W. 1977. Pollen-ovule ratios a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32–46.

DAFNI, A. 1992. Pollination ecology: a practical approach. Oxford University Press, New York, New York, USA.

DARWIN, C. 1876. The effects of cross and self-fertilization in the vegetable kingdom. John Murray, London, UK.

DING, Z., AND K. LARSEN. 2000. Taccaceae. *In* Z. Wu. and P. H. Raven [eds.], Flora of China, vol. 24, 274–276. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, Missouri, USA.

DRENTH, E. 1972. A revision of the family Taccaceae. *Blumea* 20: 367–406.

ECKERT, C. G., AND C. R. HERLIHY. In press. Using a cost-benefit approach to understand the evolution of self-fertilization in plants: the perplexing case of *Aquilegia canadensis* (Ranunculaceae). *Plant Species Biology*.

ENDRESS, P. 1995. Major evolutionary traits of monocot flowers. *In* P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries [eds.], Monocotyledons: systematic and evolution, part 1, 43–80. Royal Botanic Gardens, Kew, UK.

FÆGRI, V., AND L. VAN DER PIJL. 1971. Principles of pollination ecology, 2nd ed. Pergamon, Oxford, UK.

FENSTER, C. B., W. SCOTT ARMBRUSTER, P. WILSON, M. R. DUDASH, AND J. D. THOMSON. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology and Systematics* 35: 375–403.

FU, L.-K., AND J.-M. JIN. 1992. China plant red data book—rare and endangered plants, vol. 1, 642–643. Science Press, Beijing, China.

HARDING, J., AND K. BARNES. 1977. Genetics of *Lupinus*. X. Genetic variability, heterozygosity, and outcrossing in colonial populations of *Lupinus succulentus*. *Evolution* 31: 247–255.

JOHNSON, S. D., AND K. E. STEINER. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* 15: 140–143.

LAMBORN, E., AND J. OLLERTON. 2000. Experimental assessment of the functional morphology of inflorescences of *Daucus carota* (Apiaceae): testing the 'fly catcher effect.' *Functional Ecology* 14: 445–454.

LARSON, B. M. H., P. G. KEVAN, AND D. W. INOUYE. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. *Canadian Entomologist* 133: 439–465.

LLOYD, D. G. 1980. Demographic factors and mating patterns in angiosperms. *In* O. T. Solbrig [ed.], Demography and evolution in plant populations, 67–88. Blackwell, Oxford, UK.

LLOYD, D. G. 1987. Allocations to pollen, seeds, and pollination mechanisms in self-fertilizing plants. *Functional Ecology* 1: 83–89.

MORGAN, M. T., AND S. C. H. BARRETT. 1989. Reproductive correlates of mating system evolution in *Eichhornia paniculata* (Spreng) Solms (Pontederiaceae). *Journal of Evolutionary Biology* 2: 183–203.

ORNDUFF, R. 1969. Reproductive biology in relation to systematics. *Taxon* 18: 121–133.

PROCTOR, M., P. YEO, AND A. LACK. 1996. The natural history of pollination. Timber Press, Portland, Oregon, USA.

RITLAND, K. 2002. Extensions of models for the estimation of mating systems using *n* independent loci. *Heredity* 88: 221–228.

ROUBIK, D. W. 1989. Ecology and natural history of tropical bees. Cambridge University Press, Cambridge, UK.

SAS INSTITUTE. 2000. JMP statistics and graphics guide, version 4.0.4. SAS Institute, Cary, North Carolina, USA.

SAW, L. G. 1993. *Tacca*: flowering and fruiting behaviour. *Nature Malaysiana* 18: 3–6.

SCHOEN, D. J. 1982. The breeding system of *Gilia achilleifolia*: variation in floral characteristics and outcrossing rate. *Evolution* 36: 352–360.

STEBBINS, G. L. 1970. Adaptive radiation in angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326.

STEVENSON, D. W. 2004. Taccaceae. *In* N. Smith, S. A. Mori, A. Henderson, D. W. Stevenson, and S. V. Heald [eds.], Flowering plants of the Neotropics, 483–484. Princeton University Press, Princeton, New Jersey, USA.

WASER, N. M., L. CHITTKA, M. V. PRICE, N. WILLIAMS, AND J. OLLERTON. 1996. Generalization in pollination systems and why it matters. *Ecology* 77: 1043–1060.

WEBERLING, F. 1992. The morphology of flowers and inflorescences. Cambridge University Press, Cambridge, UK.

WENDEL, J. F., AND N. F. WEEDEN. 1991. Visualization and interpretation of plant isozymes. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 5–45. Dioscorides Press, Portland, Oregon, USA.