# Reproductive Biology, Mating System, and Population Genetics of Devil Flower: An Autonomous Selfing Plant with Showy Floral Display

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## ABSTRACT

*Tacca*, a genus of tropical herbs, possesses near black flowers, conspicuous involucral bracts and whisker-like filiform bracteoles. These unusual floral features puzzle every botanist and beg the question whether their large involucral bracts and long whisker-like filiform bracteoles play a role in pollinator attraction, or function in defense from herbivores. Recent studies of pollination, mating system and population genetics of *Tacca chantrieri* revealed that it is a highly self-pollinating species, and their showy floral structures play a limited role in pollinator attraction. This mating pattern leads to significant spatial genetic variation among populations. The population genetic structure is also determined by the population history and environmental circumstances. Significant genetic differences between two distinct geographic regions of *T. chantrieri* have been documented and might be attributable to vicariance along the Tanaka Line, as gene flow was blocked. *T. integrifolia* also possesses the same population genetic pattern. Moreover, because of their ornamental floral structure, *Tacca* plants have become increasingly popular in the horticultural trade; and some relevant studies about their seed biology and horticultural techniques have been done. Future studies about *Tacca* should focus on the origin and evolution of their bizarre floral structures and the function of natural selection on reproductive traits in natural populations.

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## INTRODUCTION

Since Darwin's pioneering studies, it has been widely recognized that out-crossing is preponderant than selfing (Darwin 1876; Charlesworth and Charlesworth 1987a). 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). On the contrary, mainly self-fertilized plants will not allocate more resources on pollinator attraction structures; instead they allocate these resources on other structures or function (Charnov 1982; Lloyd 1987). Thus according to this theory, its sexual reproduction for great extent might depend on out-crossing if the plant allocates mass resources on extravagant floral structures and display.

Taccaceae is a small family comprised 12 species (Zhang 2006). It has a pantropical distribution range centralized in Indo-Malesia (SE Asia to the Solomons), with one distributed through Asia to Australia and Africa (*T. leontopetaloides*), and another only distributed in tropical South America (*T. parkeri*) (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. 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. Several of the vernacular names used for *T. chantrieri* ("bat plant", "tiger's whisker", or "devil flower") reflect these images (**Fig. 1**). *Tacca* plants always attract numerous botanists because of their extremely variant floral display. Several studies have focused on morphology, cytology and phytochemistry.

Drenth (1972, 1976), Ling (1985) and Li (1995) had done some works on taxonomic revision of the Taccaceae, based only on the examination of herbarium specimens and traditional morphological study. However, controversies still remained in this family as to the infrageneric subdivision of *Tacca*: the recognition of *Schizocarpsa* or not, the difference within and among species, comprehensive taxonomic evidences and field observations are lacking. Referring to literature and field observation, as well as pictures and descriptions on the website, *T. chantrieri* and *T. integrifolia*, with a wide geographical distribution, exhibit large morphological differences. Especially noticeable are differ-

Invited Review

**Fig. 1 The floral characters and habitats of *Tacca* species.** Amazing different floral displays in Taccaceae. (**A**) *T. subflabellata*. (**B**) *T. chantrieri*. (**C**) *T. ampliplacenta*. (**D**) *T. integrifolia*. (**E**) *T. palmata*. (**F**) *T. leontopetaloides*. (**G**) *T. palmatifida*. (**H**) *T. parkeri*. (**A, B, E, F, G**) photos by Ling Zhang; (**C, D**) photos by Qing-Jun Li; (**H**) photos by Lisa M. Campbell.

ences of shape, color and size of bracts and plant sizes among the different regions and habitats (Zhang *et al*. unpublished data). *T. integrifolia* has a core distribution in southeastern Asia and disjunct populations in the upper Brahmaputra valley in Tibet, China. Significant morphological differences exist between these two distributional regions (Zhang *et al*. 2006b) (**Fig. 2**).

According to our preliminary comparison of morphological characters and geographical distribution, *T. integrifolia* ranges from the Brahmaputra valley south toward the Malaysia region. *T. ampliplacenta*, distributed in southwest of Yunnan, possess the same floral characters with *T. chantrieri* but its fruit structures are same as *T. integrifolia*, dis-

tinguished with intumescent placentas that fill the fruit cavity, then we thought *T. ampliplacenta* might be a substitute species (Zhang and Li, in press). Most of the family's members are native to tropical Asia, but only one species (*T. parkeri*) is distributed in tropical South America (**Fig. 1**). This poses an interesting phyto-geographical question as to the relationship between it and other species and the reason for the present distribution pattern.

The rhizome of the *Tacca* plants is used as medicine. It has medicinal virtues of detoxification, diminish inflammation and acesodyne, can cure abscesses of the stomach and duodenum, high blood pressure, hepatitis, gastralgia, scalds, burns, tumefaction and ulcers. Because of its poten-
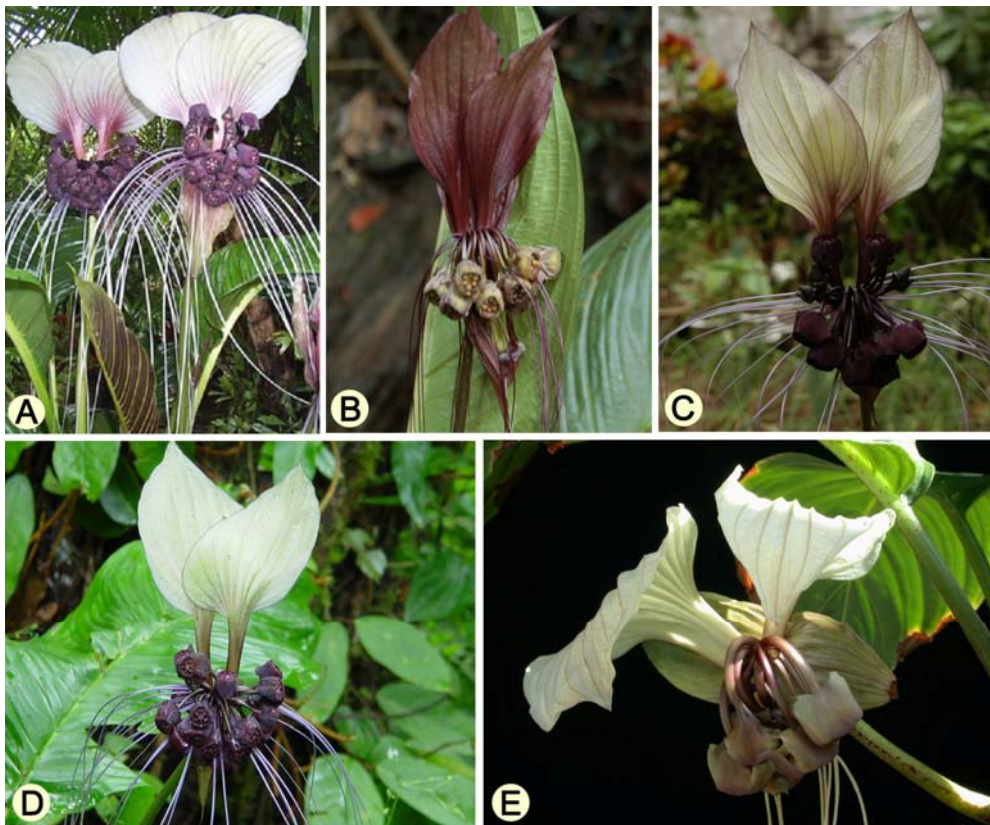
**Fig. 2 Morphological variation of bracts of *Tacca integrifolia* from different places.** (**A**) Cultivated plant (Nakara garden, Northern Territory, Australia) (Pauline Leafe). (**B**) Wide plant in Seramban, Malaysia. (**C**) Cultivated plant at XTBG (introduced from Medog). (**D**) Wide plant in Medog. (**E**) Cultivated plant in Smithsonian Institution (introduced from Myanmar, W. John Kress). (Reprinted from Zhang *et al*. 2006b, with kind permission from Science Press, Beijing).

tial medicinal benefits, phytochemistry studies had been carried out. Scheuer *et al*. (1963) found *T. leontopetaloides*, contained β-sitosterol, ceryl ethanol and one unknown compound of Taccalin. Abdel *et al*. (1990) and Abdallah *et al*. (1990) also studied *T. leontopetaloides* and *T. integrifolia*, they found compounds from these plants had very good anti-cancer efficacy.

Despite the unusual reproductive morphology of *Tacca*, there have been no detailed investigations of the pollination biology and mating system, nor on the biosystematics, reproductive biology and conservation biology of *Tacca*. The cytological studies about *Tacca* are only on two species: *T. leontopetaloides* (2n=30) (Darlington and Wylie 1955) and *T. plantaginea* (2n=20) (Ling 1985). And the study of biological characters of *Tacca* is very rare; there were just some preliminary observations and speculations (Faegri and van der Pijl 1971; Drenth 1972; Saw 1993). Recently Zhang *et al*. (2005, 2006a, 2006b) have done some work on *Tacca* pollination biology, mating system, genetic diversity and genetic structures. In this paper, we want to focus on the reproductive biology, mating system, population genetics, conservation implications and horticulture in the *Tacca* genus.

## ARE POLLINATION SYNDROMES USEFUL PREDICTORS OF FLORAL VISITORS IN *TACCA*?

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 (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 sapromiophilous syndrome (Drenth 1972; Saw 1993). Stevenson (2004) also suggested that the dark-colored flowers and large bracts were likely to be associated with fly pollination (see Endress 1995). 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). For the sake of establishing whether or not the unu-

sual 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, *T. chantrieri* populations in SW China had been selected by Zhang *et al*. (2005) to investigate pollination and mating pattern.

*T. chantrieri*, an endangered species (Fu and Jin 1992),



**Fig. 3 The distribution map of *Tacca chantrieri*.**

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inhabits moist and shaded understory habitats in tropical forests of SE Asia including Thailand, Malaysia, and southern China, particularly Yunnan Province (**Fig. 3**). Plants are 50-100 cm tall with tubers or creeping rhizomes and alternate, elliptic, entire leaves. It 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. 1**). Even though possessing a suite of traits usually associated with sapromyiophily, field investigations of *T. chantrieri* failed to reveal deceit pollination mediated by flies. Contrary to expectation, populations of *T. chantrieri* were highly selfing, and flowers have several traits that promote autonomous self-pollination.

Undoubtedly, as described by Drenth (1972) and Saw (1993), species of *Tacca*, including *T. chantrieri*, possess a suite of traits normally associated with sapromyiophily. Usually 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 in Xishuangbanna area there were no any odor associated with the flowers or bracts of *T. chantrieri* to be detected, and in the upper Brahmaputra valley in Tibet China, *T. integrifolia* possess rather showy floral structures, we also failed to see any pollinators visit their flowers and could not detect any musky smell in *T. integrifolia* (Zhang *et al*. unpublished data). The same traits occur in *T. subflabellata* and there were no pollinator visits and bagged inflorescences set fruits (Zhang *et al*. unpublished data). Perhaps *Tacca* produce compounds attractive to flies but not perceptible by humans, as is the case in the fly-pollinated *Asarum* (Proctor *et al*. 1996). However, analysis of the atmosphere around the flower of *T. chantrieri* showed that only limonene and linalool were slightly different from the ambient air, furthermore the amount of these compounds were very low (Zhang *et al*. unpublished data).

Moreover, with the possession of traits commonly associated with fly pollination by deceit, no flies were recorded acting as pollinators in *T. chantrieri*. The only pollinator observed was *Trigona*, a ubiquitous pollen-collecting tropical bee that visits many species opportunistically (Roubik 1989). Pollinator visitation was infrequent. The 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 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. The finding of similar levels of seed set in bagged and open-pollinated flowers at one population (although not at another population) implies that most selfing probably arises by autonomous means. Selfing rates estimated in four populations using allozyme makers averaged 0.86 (range 0.76-0.94), thus corroborating this inference. The results indicate that despite considerable investment in extravagant display, populations of *T. chantrieri* are highly selfing (Zhang *et al*. 2005).

The floral biology and mating system of *T. chantrieri* indicate that most seeds produced in populations result from self-fertilization. The low pollen vs. ovule ratios (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). These floral structures were common in several species in *Tacca*. Pollinator-facilitated, intra-floral and inter-floral (geitonogamous) selfing may also contribute to high selfing rates in

populations visited by pollinators. Why does *Tacca* 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 inter-floral selfing. Three hypotheses had been proposed to explain the association between the sapromyiophilous syndrome and high selfing rates in *Tacca*.

**First, mating patterns may have spatial and temporal variation** – A 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. 3**). 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, many studies showed that reduced investment in both floral and inflorescence display is usually associated with predominant selfing (Ornduff 1969; Charnov 1982; Schoen 1982a; Lloyd 1987; Morgan and Barrett 1989). Apparently, this has not occurred in *T. chantrieri* despite mechanisms for autonomous selfing and the low pollen vs. ovule ratio of the species.

The mating patterns in the populations of *T. chantrieri* investigated could fluctuate from year to year and 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 2004).

**Second, 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 lesser extent bracteoles, would involve significant remodeling 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. The autonomous selfing mechanisms and low pollen: ovule ratio of *T. chantrieri* are certainly consistent with this scenario; however, the sub-maximal fruit set of open-pollinated and bagged flowers are not expected in populations with a long history of selfing. In most autogamous plants, flower to fruit ratios are close to 1.0, suggesting that the reproductive economy of the *T. chantrieri* populations that been investigated may not be fully adjusted to high levels of selfing.

The 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 (bracts removed and fixed) was not significantly

different from the control treatment. Several deficiencies in the experiment may have prevented detecting significant treatment effects: e.g., no attempt to measure visitation rates because pollinators were so infrequent at the site, therefore, a lack of information on whether manipulations influenced pollinators in any way; also, only maternal reproductive success (fruit and seed set) in the treatments were compared and not male fitness resulting from pollen dispersal between plants; finally, the manipulations were not conducted at the whole plant level, and the inflorescence level modifications may have been insufficient to illicit differential responses. Nevertheless, despite these shortcomings and given the 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.

**Third, 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. 1**). Previous workers have assumed that these structures function to attract flies to plants in the shaded understory of tropical forests. Given their leaf-like 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 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.

## POPULATION GENETICS OF TWO SPECIES OF *TACCA* WITH SHOWY FLORAL STRUCTURE

The spatial distribution of genetic diversity in plant populations, which is characterized as the genetic variability and genetic differentiation within and among populations, is primarily determined by plant life history including reproductive traits (Schoen 1982a, 1982b; Schoen and Clegg 1985; Hamrick and Godt 1996), but population history is also a determinant factor of the genetic variation within species (Schaal *et al*. 1998). Population history, represented as fluctuations in the number and size of populations, and the evolutionary and biogeographic histories of species, may have played critical roles in determining its current genetic composition (Schaal *et al*. 1998). Contemporary biogeographic patterns of genetic variation are determined by historical patterns of gene flow and vicariance among populations (Hewitt 1996; Soltis *et al*. 1997; Avise 2000). This history should be reflected in the genetic structure and phylogeography of extant populations, which would provide information for inferring evolutionary processes and for testing biogeographical scenarios that underlying patterns of genetic differentiation. As such, if the population genetic structure of a species is understood, its evolutionary history can be elucidated (Bauert *et al*. 1998).

Plant reproductive traits also determine population genetic structure via the influence of a plant's mating system (Hamrick and Godt 1990; Schoen *et al*. 1996). The close relationship between the mating system and the level of genetic variation and genetic structure has been documented in many studies using different methods (Brown *et al*. 1989; Hamrick and Godt 1990). Inbreeding species are expected to have less genetic diversity and heterozygosity within populations, as well as more genetic differentiation among populations, than outcrossing species (Charlesworth and Charlesworth 1995; Hamrick and Godt 1996). Therefore, genetic diversity within and among populations can reflect, to a certain extent, the relative rates of inbreeding vs. outcrossing in a species.

As an important reproductive trait, floral design and display affects plant mating systems by attracting animal pollinators and thereby promoting pollen dispersal and cross-pollination (Harder and Barrett 1996; Emms *et al*. 1997). It is believed that plants with greater investment in floral structures attractive to pollinators will benefit from increased fitness via cross-pollination. In this case, species with a high investment in extravagant floral displays are expected to be largely outcrossing (Charlesworth and Charlesworth 1987b). So if *T. chantrieri* possess considerable spatial or temporal variation in outcrossing rates among populations, then it will influence the genetic structure of the populations. Zhang *et al*. (2006a) investigated whether genetic variability and genetic differentiation occurred within or among populations using Inter-Simple Sequence Repeats (ISSR).

The survey of 14 populations of *T. chantrieri* revealed a high level of genetic variation at the species level, but there was considerable variation (**Table 1**). This implied that a large proportion of genetic variation was partitioned among populations. In general, selfing species usually possess lower genetic diversity within populations and higher genetic differentiation among populations relative to outcrossing species (Hamrick and Godt 1996). Therefore, the data on the population genetic structure in *T. chantrieri* at large spatial scales are consistent with the highly selfing mating system documented at smaller spatial scales (Zhang *et al*. 2005). Among these 14 populations, the mating systems of two populations (WEV and BB) have been quantified by allozyme markers in our previous research. The WEV population had the highest selfing rate ($S_m = 0.941$), and exhibited the lowest genetic diversity ($PPB = 15\%$, $H_E = 0.041$). The BB population also had a quite high selfing rate ($S_m = 0.859$) and contained very low genetic variation ($PPB = 25\%$, $H_E = 0.079$) (Zhang *et al*. 2005). Among the four populations of *T. chantrieri* examined in the previous study, estimates of the population level maternal selfing rate ($S_m$) averaged 0.86 (range 0.76-0.94). This is a high figure, and is similar to that of other obligately selfing species. Consistent with this, the average genetic diversity in the south Yunnan–Thailand region was very low ($PPB = 23.125\%$, $H_E = 0.075$).

Comparing with genetic variation and structure based on RAPD analyses of other wild plant populations (Nybom and Bartish 2000; Nybom 2004), the amount and pattern of genetic variation in *T. chantrieri* is more comparable to selfing or mixed mating taxa than to outcrossing species. The $G_{ST}$ among populations was 0.5835, which was similar to the average for selfing plant species (0.51) in Hamrick and Godt's (1990) analysis. A high level of population differentiation may be explained by several factors, such as the species' breeding system, genetic drift, demographic fluctuations, or the genetic isolation of populations (Hogbin and Peakall 1999). When populations are small and geographically and genetically isolated from one another, genetic drift influences the genetic structure and increases differentiation among populations (Barrett and Kohn 1991; Ellstrand and Elam 1993). Estimates of the effective gene flow per generation ($Nm$) of *T. chantrieri* were lower (0.3568) than one successful migrant per generation. This indicated limited gene flow among populations, which may be insufficient to counteract the effect of genetic drift. While inferences of the migration rate from estimates of $Nm$ are not definitive for populations that do not exhibit metapopulation dynamics or large demographic shifts (Whitlock and McCauley 1999), the method is still a reasonable guide to levels of gene flow among populations. The low estimates of migration among *T. chantrieri* populations correspond

**Table 1** Pooled values and mean genetic variabilities within populations of *T. chantrieri* and *T. integrifolia* detected by ISSR analysis.

| | Population | $N$ | $H_E$ | PPB (%) | $G_{ST}$ | $Nm$ | $F_{ST}$ | Among populations | Within populations |
|---|---|---|---|---|---|---|---|---|---|
| West region | BSH | 20 | 0.098 | 27.5 | | | | | |
| | QCT | 22 | 0.111 | 35 | | | | | |
| | BB | 20 | 0.079 | 25 | | | | | |
| | Th | 20 | 0.034 | 8.75 | | | | | |
| | WEV | 20 | 0.041 | 15 | | | | | |
| | GLQ | 21 | 0.085 | 27.5 | | | | | |
| | Mean | | 0.075 | 23.13 | | | 0.6678 | 66.78 | 33.22 |
| | Pooled | 123 | 0.168 | 71.25 | 0.563 | 0.388 | | | |
| East region | MLP | 21 | 0.156 | 41.25 | | | | | |
| | NWH | 15 | 0.09 | 27.5 | | | | | |
| | TP | 20 | 0.181 | 55 | | | | | |
| | DL | 35 | 0.165 | 50 | | | | | |
| | CZ | 16 | 0.104 | 28.12 | | | | | |
| | PS | 25 | 0.151 | 40.62 | | | | | |
| | WN | 23 | 0.16 | 45 | | | | | |
| | QZ | 25 | 0.095 | 31.88 | | | | | |
| | Mean | | 0.138 | 39.92 | | | 0.5601 | 56.01 | 43.99 |
| | Pooled | 180 | 0.234 | 77.5 | 0.4168 | 0.6997 | | | |
| | Average | 21.6 | 0.165 | 32.72 | 0.5835 | 0.3568 | 0.6829 | 69.89 | 30.11 |
| | Species level | | 0.264 | 90.62 | | | | | |
| *Tacca integrifolia* | JX | 19 | 0.01 | 3.66 | | | | | |
| | MD | 23 | 0.034 | 8.54 | | | | | |
| | BB | 23 | 0.028 | 7.32 | | | | | |
| | Ma | 19 | 0.103 | 31.72 | | | | | |
| | Average | 21 | 0.065 | 12.81 | 0.145 | 2.948 | 0.9206 | 92.06 | 7.94 |
| | Species level | | 0.185 | 67.68 | | | | | |

$N$, sample size; $H_E$, expected heterozygosity; *PPB*, percentage of polymorphic loci. $G_{ST}$, genetic differentiation between populations; *Nm*, estimated gene flow; $F_{ST}$, genetic differentiation between populations.

well with the geographic isolation of the populations, in which genetic differentiation among populations appears to be correlated with geographic distance between populations.

The relationship analysis between the genetic distances among populations and their geographic distances (**Fig. 3**) revealed that significant genetic differences between the south Yunnan–Thailand and southeast Yunnan–Guangxi–Hainan populations, which are coincidently separated by a presumed biogeographic boundary, the Tanaka line (Tanaka 1954; Li and Li 1997). Current genetic diversity distribution pattern of *T. chantrieri* populations might be due to a possible evolutionary event under vicariance from a single common ancestor through fragmentation of its original geographic range, and this vicariance could be explained by the different history of the geological structure on each side of the Tanaka line.

The Tanaka line is considered to be a boundary between the Sino-Japanese plate/biogeographic region in the east and the Sino-Himalayan plate/biogeographic region in the west. The approximate position of the Tanaka line can be shown as a straight line starting at the intersection of 28ºN, 98ºE southward to approximately 18º45' or 19ºN, 108ºE (**Fig. 3**). In general, the flora components of the Sino-Japanese region are relictual and the elements of the Sino-Himalayan are evolved. The southeast Yunnan–Guangxi–Hainan area is part of an important floristic region in China called the Dian–Qian–Gui biogeographic region and is located on

the east side of the Tanaka line. This region is noted for species abundance, endemism and historically high rates of speciation. The results indicated that expected heterozygosity of *T. chantrieri* was higher to the east ($H_E = 0.234$) than to the west ($H_E = 0.168$) of the Tanaka line (**Table 1**). However, genetic differentiation among populations was greater to the west ($Gst = 0.563$, $F_{ST} = 0.6678$) than to the east of the line ($Gst = 0.417$, $F_{ST} = 0.5601$) (**Table 2**). All these observations are consistent with an evolutionary origin for *T. chantrieri* in the Dian–Qian–Gui region, with a relatively recent range expansion to the west, resulting in reduced diversity and a higher population differentiation in western region.

The genetic structure of plant populations is also influenced by the long-term evolutionary and ecological history of the species, which would include shifts in distribution, habitat fragmentation, and population isolation (Schaal *et al*. 1998). Wu *et al*. (2003) proposed that *Tacca* originated from the southern marginal area of the Palaearctic continent when Pangaea expanded to the Pacific Ocean for the first time. Later, this genus became differentiated in a succession of nearby environments. Moreover, they also hypothesized that the northern part of the Indo-Chinese peninsular, stretching from Yunnan to Tibet, might be the site of ancient differentiation of this genus. The pattern of genetic structure of *T. chantrieri* is consistent with this hypothesis, the genetic diversity was quite high in southeast of Yunnan (TP and

**Table 2** Analyses of variance conducted with different life history traits as group variable, and with mean STMS-drived estimated of within-population gene diversity $H_E$ and $H_O$ and among-population differentiation $G_{ST}$ or $F_{ST}$ as dependent variables. (Derived from Hamrick and Godt 1996; Nybom 2004).

| Variable | N | $H_E$ | N | $H_O$ | N | $G_{ST}$/ $F_{ST}$ |
|---|---|---|---|---|---|---|
| Annual long-lived perennial | 59 | 0.68 | 55 | 0.63 | 17 | 0.19 |
| Widespread species | 31 | 0.62 | 23 | 0.57 | 13 | 0.25 |
| Selfing | 15 | 0.41 | 4 | 0.05 | 5 | 0.42 |
| Mixed | 15 | 0.6 | 13 | 0.51 | 5 | 0.26 |
| Outcrossing | 71 | 0.65 | 60 | 0.63 | 23 | 0.22 |
| Selfing widespread | 38 | 0.165 | | | | 0.446 |
| Annual selfing | 102 | 0.131 | | | | 0.553 |
| Regional annual long-lived perennial | 151 | 0.190 | | | | 0.086 |
| *Tacca chantrieri* (Average/species) | 14 | 0.165/0.264 | | 0.111/0.402 | | 0.59/0.68 |
| *Tacca integrifolia* (Average/species) | 4 | 0.065/0.185 | | 0.044/0.292 | | 0.15/0.92 |
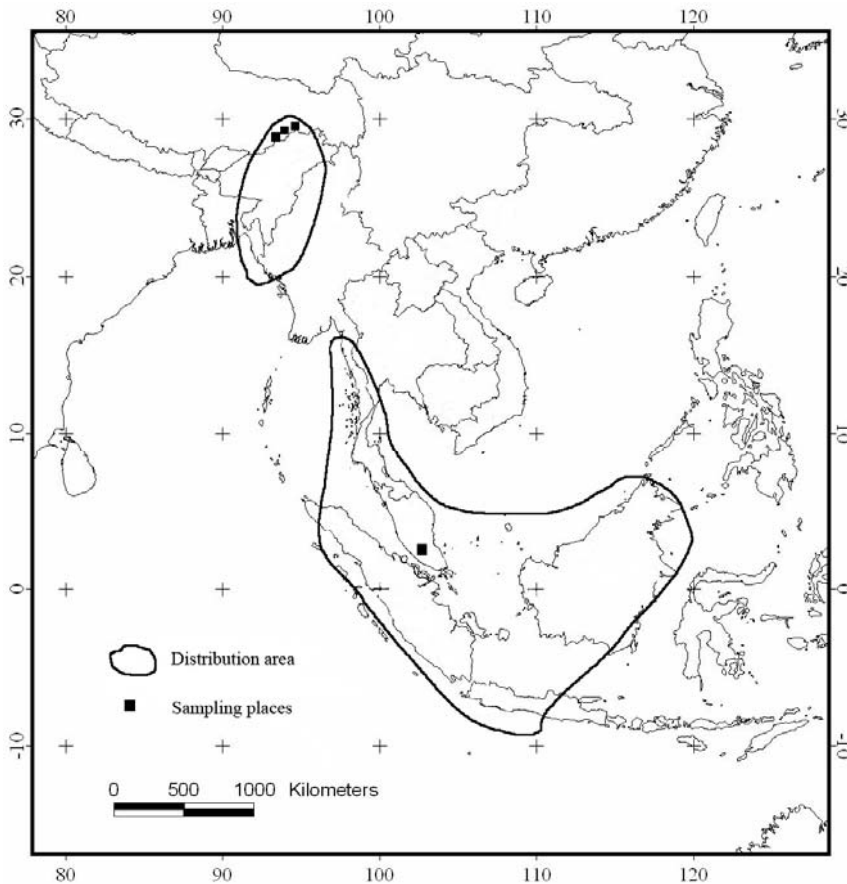
**Fig. 4 Map showing the distribution and sampling sites of *Tacca integrifolia.***

MLP populations), Guangxi (DL and PS populations), and Hainan (WN population) areas. This geographic area corresponds exactly to one of the centers of endemism in China (southeast of the Yunnan, Guizhou, and Guangxi regions) (Li 1996) and Hainan, which became separated from the south of China five million years ago (Zhu and Roos 2004). This investigation supports Wu's hypothesis to a certain extent, the original geographic region of this species lies from Vietnam to the northern edge of the subtropics in southeast Yunnan, Guizhou, Guangxi, and Hainan (the Dian –Qian–Gui region) (Wu *et al*. 2003). At the very least, the populations on the east side of the Tanaka line originated earlier than those on the west side. This genetic structure of *T. chantrieri* is unexpected for a species with an extravagant floral display, but corresponds with the mating system of this species as previously quantified, indicating that *T. chantrieri* populations are predominantly selfing. Geographical patterns of genetic differentiation of *T. chantrieri* provide strong evidence for its evolutionary and ecological histories, and the vicariance among populations.

Another species of *Tacca*, *T. integrifolia*, has a core distribution in southeastern Asia and disjunct populations in the upper Brahmaputra valley in Tibet, China. It also has very showy floral structures. With a rich investment to floral structures to attract pollinators and thus achieve outcrossing, a high genetic diversity was expected. *T. integrifolia* is mainly distributed in Pakistan, Sri Lanka, East India, Combodia, Laos, Burma, Thailand, Vietnam, Malaysia and Indonesia (Drenth 1972) (**Fig. 4**). It occurs from sea level up to 1200 m, rarely to 1500 m altitude. It has a broad distribution range and wide latitude span, but there is big geographical disjunct between south and north distributing regions (**Fig. 4**).

*T. integrifolia* grows mostly in evergreen moist primary or secondary forests, e.g. on steep slopes, ridges, or near water, sometimes on roadsides and in clearings. It was found on various soils, on sandy or stony substrata, limestone or red earth. Specimens from the hills are claimed to be larger than those from the plains (Drenth 1972). In China,

it just grows under tropical virgin forests in southeast of Tibet, mainly distributes in Medog Brahmaputra valley, 600-850 m in altitude, and is dominated by *Altingia excelsa* (Hamamelidaceae) and *Terminalia myriocarpa* (Sapindaceae) (Sun and Zhou 2002). This region is the northern boundary of this species. There should not be any tropical forest at this latitude, but due to its special topography, this region possesses tropical local microclimates, especially in south-toward-north valleys and alpines, some tropical elements distributed here along the valley. Some tropical plants in this region are separated with their core distribution, and go with alternate glacial – interglacial in the endless geological history, then their distribution range shifted back and forth, these will certainly cause the changes in population genetic structure (Hewitt 2000). Is *T. integrifolia* such a case?

Significant morphological differences exist among different distributions of *T. integrifolia* due to long-term geological separation (**Fig. 2**). Do these kinds of morphological differences reflect its genetic differentiation? If so, what are the levels of genetic differentiation among and within populations in this species? The ideal study needs to detect most populations from the whole distribution range, but this work has not been done. Fortunately, genetic variation within and among four populations were assessed (three from the upper Brahmaputra valley, and one from Malaysia).

Geographical distribution is one of the key factors that determine plant genetic diversity. In general, plants possess a higher level of genetic diversity if they have relatively wider distribution range (Korron 1987; Hamrick and Godt 1990). *T. integrifolia* has wide range of distribution, so higher genetic variation was expected. The result indicated that at species level, genetic diversity of *T. integrifolia* was high, while at population level, diversity was low, especially in the Brahmaputra populations (**Table 1**). Extremely high levels of genetic differentiation were detected among populations based on different genetic diversity analyses. At the population level, all parameters of diversity were lower than that of self-pollinated plants which Nymbon proposed

(2004). Moreover, the genetic diversity of Medog populations was much lower than that of Malaysia.

The genetic variation within the populations was very low and very high between populations ($G_{ST}$= 0.777, $F_{ST}$= 0.9206, $N_m$= 2.948). Such value was almost 3.4 time higher than the average (0.228) of woody plants (Hamrick *et al*. 1992), and was also higher than the average of selfing plants ($G_{ST}$ = 0.59) (Nymbon 2004) (**Table 2**). Many studies have shown that population genetic structure is capable of reflecting the reciprocity of different processes among plants long-term evolutionary history (distributing area shift, fragmentation of habitats and population specialization), mutation, genetic drift, mating systems, gene flow and genetic isolation among populations. By contrast, a high level of population genetic differentiation could be the result of breeding system, genetic drift and genetic isolation among populations (Slatkin 1987; Schaal *et al*. 1998; Hogbin and Peakall 1999). Wright (1951) pointed out that genetic drift could lead to the obvious genetic differentiation among populations if $N_m$ < 1. Medog population was about 2000 km away from Malaysian population, and $N_m$ was 0.1435.

Restricted gene flow among populations of *T. integrifolia* may result from several factors, such as self-pollination, inefficient seed dispersal, isolation of populations and fragmentation of habitats. The extremely low genetic diversities within Brahmaputra populations are probably due to the special topography of the Brahmaputra valley and vicariance.

Generally, the breeding system of flowering plant species greatly affects population genetic differentiation. Selfing can result in low genetic diversity within populations. Hamrick *et al*. (1991) indicated that the genetic diversity among selfing populations was 5 times higher than outcrossing species, but it would reduce to half level than that of outcrossing species within populations. Even though nothing is known about the mating system of *T. integrifolia*, and the reality of pollinator shortage leading to selfing is need to be approved, we still can speculate on its mating system from some biological characters and geographical distribution pattern. *T. integrifolia* has a very showy floral structure, suggesting that its exaggerated floral resources investment is supposed to attract insects and favor outcrossing; therefore we presume that it should possess high genetic diversity. However, Medog populations have a very low genetic variation; Malaysian populations also possessed relatively low genetic variations. How to explain this phenomenon?

The floral biological characteristics of *T. integrifolia*, e.g. lack of fragrance, no nectar secretion which most pollinators like, close proximity of stigma and stamen, no effective pollinators (Zhang *et al*. unpublished data), are regarded as adapting to self pollination. Then there is no wonder as to the failure of multi-locus selfing rates estimated in 3 populations using allozyme markers (Zhang *et al*. unpublished data). As such, *T. integrifolia* is also supposed to be a self-pollinated species compared with the previous study in *T. chantrieri* (Zhang *et al*. 2005), not less than the Medog populations.

Generally speaking, plant geographical distribution is considered to agree with the biogeographic models, which predict that geographically peripheral populations should be smaller, more sparely distributed and less productive than populations near the center of a species' range (Hengeveld and Haeck 1982; Brown 1984; Lawton 1993; Sagarin and Gaines 2002). Plants in peripheral populations, therefore, may receive less pollinator visitation and outcross pollination, which may cause selection for self-fertilization to provide reproductive assurance. The Medog populations of *T. integrifolia* have the extremely low variation that may be due to their furthest north distribution range. The glacial refugium hypothesis (GRH) proposes that glaciers promoted differentiation and generation of intraspecific diversity by isolating populations in ice-free refugia (Holder *et al*. 1999). Climatic oscillations through the Tertiary (65 million years (Myr)) with frequent oscillations that increased in ampli-

tude and led to the series of major ice ages of the Quarternary (2.4 Myr to the present) produced great changes in species distributions. Tropical rainforests were restricted and dissected, species went extinct over large parts of their range, some dispersed to new locations, some survived in refugia and then expanded again. This must have occurred repeatedly (Hewitt 2000). With repeated climatic oscillations and range changes, a population may have passed through many such adaptations and reorganizations and its genome structure could diverge considerably.

The Eastern Himalaya is one of the 25 global (one of the two in the northern hemisphere) biodiversity 'hotspots' (Myers 1988; Wilson 1992; Myers *et al*. 2000). The area comprises Nepal, Bhutan, and neighboring states of northern India along a continuous sector of the southeast Tibet, southwest Sichuan, and northwest Yunnan provinces of China. This region is the meeting ground of the Indo-Malayan, Indo-China, and eastern Asian biogeographical realms following the formation of the Himalayas, which resulted from the collision of the India plate with the Laurasia landmass (Behera *et al*. 2002). Studies have shown that this region was a centre of active speciation and a refugium for various flowering plants during glacial maxima (Takhtajan 1969; Rao 1994). Due to the complicated topography of the areas and the low accessibility, genetic communication with other area is impossible for plants in this region. Populations of *T. integrifolia* in this region have extremely high levels of population genetic differentiation comparing with its core distribution; this may result from self-pollination, inefficient seed dispersal, population isolation, and habitat fragmentation. The extremely low genetic diversity in Brahmaputra populations is probably due to the unique landforms and vicariance of the Brahmaputra valley. Moreover, significant morphological differences exist between the two regions. The color and shape of bract of this species varies in different geographical distributions. Phylogenetic study of *Tacca* genus based on *trn*L-F also showed that the Medog population of this species comprised ca. 200 bp shorter sequence than that in Malaysian population (Zhang *et al*. unpublished data). A genetic study at the population level for *Tacca* genus will provide insights into both historical processes and ongoing evolutionary mechanisms in maintaining the extraordinarily high biodiversity in this region. Therefore, it may be possible to confirm that plants of *T. integrifolia* in Brahmaputra valley, already differentiated from other regions, may have developed to a new species during the long-term evolutionary processes if wider investigation of its range, morphological characters and genetic studies could be done.

## ORNAMENTAL USES OF *TACCA*

*Tacca* is a genus of tropical herbs that has conspicuous involucral bracts and whisker-like filiform bracteoles. These unusual floral features puzzle not only botanists who first encounter them in tropical understory, but also horticulturists and anthomania who collect unusual plants. The shape and color of bracts, as well as long, whisker like bracteoles in *Tacca* show diverse variation (**Fig. 1**), which are the best characters for horticulture. The whole inflorescence of *T. chantrieri* looks like a "Face of Tiger" or a "Bat" from its shape and color. *T. leontopetaloides* possesses green filiforms and lion-head-like inflorescences with hair disheveled. *T. integrifolia* has white or dark purple bracts and is fancied by people because of its rabbit-ear-like shape. *T. subflabellata* features two huge fan-like bracts and the longest bracteoles in this genus (ca. 60 cm). *T. plantaginea* is very short and tiny; every floral structure is very small and un-showy. Some other species from Southeast Asia, e.g. *T. palmate* and *T. palmatifida*, lost their filiform bracteoles, and bracts and leaves are alike, but they are good ornamental plants for their leaves. The most special species in the genus is *T. parkeri* from tropical South America which is the only species located outside of Southeast Asia. Its bracts curl up and resemble a ballerina; some individuals have lost

their bracteoles. When not flowering, the foliage is also very pretty, with dark brown petiole and long leaves looking either like pendent Bird's nest Anthuriums or small palms, depending on the species. It is very rare that the inflorescences and floral structures vary so diversely in such a small genus.

More and more flower trade companies over the world have paid more attention to this genus. In China, a lot of flower companies in the south have already cultivated plants of *T. chantrieri* as a cutting flower, especially in Yunnan, Guangdong, Hainan, etc. In the world horticulture exhibition at Kunming in 1999, *T. chantrieri* was awarded the gold medal in the flower show. Some enthusiasts also show their interests on the genus via the web. Information about the culture techniques can be found in websites such as http://www.sleepyoaks.com/batflower_faq.htm, http://www.calyx.com.au/tacca.html, http://members.tripod.com/asia_flora/tacca.htm, and so on.

Some seed biology and *in vitro* propagation of *Tacca* had been studied in recent years (He *et al.* 2002; Wen *et al.* 2002; He *et al.* 2003). For example, tissue culture for multiplying plants of *T. chantrieri* had been studied in Xishuangbanna. In this experiment, young leaves and leaf stalks obtained from seedlings were used, studies found the appropriate media for respective culture stages were as follows: The medium of seed germination was MS + 6-BA 1 mg/L + NAA 0.1 mg/L, that of callus inducing was MS + 6-BA 1 mg/L + 2,4-D 1.5 mg/L + KT 0.2 mg/L, that of shoot inducing was MS + 6-BA 0.5 mg/L + NAA 0.5 mg/L, that of rooting was 1/2 MS + NAA 0.5 mg/L (He *et al.* 2002). Wen *et al.* (2002) studied the effects of illumination, temperature and soil moisture content on germination and storage of seeds of *T. chantrieri*. Results indicated that its seeds were light-favored, germination took place at a narrow temperature range and high soil moisture content, optimum temperature was 25-30°C, the optimum soil moisture content 60-70%, and that dry storage was better way than ordinary storage for maintaining seed viability. He *et al.* (2003) found the same results, but they found that light intensity and time had no effect on the germination and different temperature caused remarkable differences to germination percentage, but in a natural condition the germination percentage stayed stable, and seeds can keep for more than a year if it keeps in 4°C. In fact, it is easy grow them from seeds and easy to manage. We have successfully grown *T. chantrieri*, *T. integrifolia*, *T. palmata* and *T. plantaginea* from seeds in Xishuangbanna Tropical Botanical Garden.

From field expeditions, we found out natural hybridization may be a common phenomenon among *Tacca* species in natural environments (Zhang *et al.* unpublished data). At least two different *Tacca* species have hybridized in Yunnan, including a North Vietnam relative, *T. subflabellata*. Hybridization has increased the genetic variation of *Tacca* in Yunnan, a new species *T. ampliplacenta* possess potential outcrossing advantage because it cannot set fruit in the greenhouse (Zhang *et al.* unpublished data). So it will be a great event if we can hybridize some species to increase their ornamental values in horticulture, such as change their bracts to be more attractive and colorful, and alter their bracts shape.

## CONCLUSION AND FUTURE PERSPECTIVES

As a result of potential medicinal and commercial values of *Tacca*, and its natural habitats rapidly disappearing, *Tacca* became an endangered species (Fu and Jin 1992). It is essential to conduct detailed studies on the pollination biology, mating system, biosystematics, and conservation biology and also horticultural techniques of cultivation and hybrid breeding for their bizarre conspicuous bracts and filiform bracteoles.

The similarity among species of *Tacca* and their Pan-tropical distribution may testify to its old origin, but nothing is known about where and when they originated. In the future, the phylogeny of the genus should be constructed to rebuild the evolutionary history of the reproductive characters in *Tacca*, and match these floral characters into a phylogenetic tree, then explore the origin and evolution key characters of reproduction. Moreover, a study of the function of natural selection on reproductive traits in natural populations of *Tacca* also needs to be done because of a mismatch between floral morphology and their mating system. As such, we may find out if long-term natural selection is capable of adjusting their patterns of resource allocation.

## ACKNOWLEDGEMENTS

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