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·**Short Communication**·

A Preliminary Study on Conservation Genetics of Three Endangered Orchid Species

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The Orchidaceae is the largest families of flowering plants with approximately 20 000 species, growing in all terrestrial ecosystems except the poles and extremely dry desserts[1]. In recent decades, however, dramatic loss of habitats, especially in the tropical and subtropical areas where most orchid species occur, tremendously threatened the lives of many orchid species[1, 2]. In addition, orchid species are always pursued by amateurs and growers from all over the world because of their spectacular and fragrant flowers[1, 2]. Thus to study, conserve and, at last, sustainably use these treasures is an urgent task facing us. Although scientists have proceeded many studies on the population dynamics and reproductive features of many orchid species[1, 3 – 5], very little is known about their levels and patterns of genetic variation, particularly at DNA level[6].

Paphiopedilum micranthum, *P. malipoense* and *Changnienia amoena* are three endangered orchid species distributing mainly in China[2]. In the present study, we investigated the genetic diversity and population genetic structures of the three species using RAPD technique. These results should contribute to a better understanding of the genetic profile of these species and could be important in developing strategies for its conservation and sustainable utilization.

1 Materials and Methods

During 1999 and 2001, 4 populations of *P. micranthum* Tang et Wang, 2 populations of *P. malipoense* S. C. Chen et Tsi and 11 populations of *C. amoena* Chien were collected in China. The size and locality of each population are listed in Table 1. Leaves were harvested and stored with silica gel in zip-lock plastic bags.

DNA extraction followed Xie *et al*[7]. PCR amplification used the same system as Qian *et al*[8]. Twelve RAPD primers were selected for *Paphiopedilum* spp, while 16 primers were selected for *C. amoena*. Genetic parameters including the percentage of polymorphic band (P), expected heterozygosity (h), and Shannon index (I), were calculated by the computer program POPGENE[9]. AMOVA was used to analyze genetic variance components and their significance levels among and within populations[10].

Table 1 Population number (Pop. no.), sizes and localities of three orchid species

Species	Pop. no.	Location	Sample size
Paphiopedilum micranthum	Pal	Anlong, Guizhou	43
	Pfn	Funing, Yunnan	17
	Pch	Ceheng, Guizhou	48
	Pwm	Wangmo, Guizhou	53
P. malipoense	Pm1	Anlong, Guizhou	3
	Pm2	Wangmo, Guizhou	7
Changnienia amoena	JGS	Jigongshan, Henan	8
	LS	Lushan, Jiangxi	25
	SNJ1	Shennongjia, Hubei	13
	SNJ2	Shennongjia, Hubei	18
	SNJ3	Shennongjia, Hubei	24
	TTZ	Tiantangzhai, Anhui	25
	XN1	Xinning, Hunan	17
	XN2	Xinning, Hunan	23
	XN3	Xinning, Hunan	23
	XN4	Xinning, Hunan	32
	XN5	Xinning, Hunan	20

2 Results and Discussion

Twelve RAPD primers produced 131 bands in *Paphiopedilum* spp. For *P. micranthum*, the percentage of polymorphic bands (PPB) was 71.6 %, Nei's gene diversity (h) was 0.217 and Shannon index (I) was 0.330 at species level. At population level, however, the means of three indexes (PPB, I and h) were 45.2 %, 0.146 and 0.220, respectively. AMOVA showed that 20.3% of the genetic diversity resulted from differentiation among populations with remaining 79.69% residing within populations. Much lower genetic diversity in *P. malipoense* was found at both species and population levels (Table 2). Sixteen RAPD primers produced 119 bands in *C. amoena*. At species level, PPB was 76.5 %, h and I were 0.194 1 and 0.305 8, respectively. At population level, the means of three indexes were 37.2%, 0.119 7 and 0.181 0, respectively. AMOVA showed that 45.27% of the genetic diversity existed among populations. A summary of genetic parameters of the three orchid species was shown in Table 2.

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Table 2 A comparison of genetic parameters of the three orchid species

	Genetic parameter	*Paphiopedilum micranthum*	*P. malipoense*	*Changnienia amoena*
Genetic diversity	Species level *PPB* (%)	71.6	49.5	76.5
	Population level *PPB* (%)	45.1	12.7	37.2
	Species level *h*	0.217 1	0.117 4	0.194 1
	Population level *h*	0.145 7	0.048 6	0.119 7
	Species level *I*	0.330 1	0.176 4	0.305 8
	Population level *I*	0.220 4	0.071 2	0.181 0
Population genetic structure	Variance component among populations (%)	20.31	–	45.27
	Variance component within populations (%)	79.69	–	54.73

Up to date, genetic diversity of a number of orchid species has been assessed by allozyme technique[11, 12], but only a single survey was conducted on any orchid species using RAPDs[6]. Wong and Sun[6] used allozyme and RAPD techniques to investigate the population genetics of a self-compatible orchid species (*Goodyera procera*)[6]. They found that there existed much higher diversity detected by RAPDs ($P = 97.0\%$, $h = 0.293$ at species level, and $P = 55.1\%$, $h = 0.181$ at population level) than that by allozymes ($P = 33.3\%$, $h = 0.151$ at species level, and $P = 21.8\%$, $h = 0.073$ at population level). Because of the lower allozyme diversity compared with many other orchid species, Wong and Sun[6] concluded that *Goodyera procera* possessed relatively low genetic diversity. Evidence showed that *Paphiopedilum* species were predominantly insect-pollinated outcrossers[13] and *C. amoena* was a self-compatible outcrosser[14]. Therefore, the three species under present study maintained lower genetic diversity in comparison with other orchid species with similar life history characteristics.

Bussell[15] reviewed RAPD studies on population genetics of 38 plant species and indicated that the average genetic variance component among populations of the 30 outbreeding species given by AMOVA was 14.4%[15]. In comparison, *P. micranthum* and *C. amoena* (with among population diversity being 20.3% and 45.3%, respectively) maintain significantly higher population differentiation than other outcrossing orchid species.

The relatively low genetic diversity and high genetic differentiation occurred in these three species are most likely to be a result of habitat fragmentation. Recent investigations indicated that the habitats suitable for orchid species in China have been seriously destroyed and fragmented[2, 3]. As a result, the number and sizes of the extant populations have decreased greatly, which in turn lead to further loss of genetic diversity and alternation of population genetic structure[16]. In addition, over collection of wild individuals is also an important factor for the rare and endangerment of orchid species, particularly for those *Paphiopedilum* species[3].

These findings, combined with other information about *P. micranthum*, *P. malipoense* and *C. amoena*, provide important basis in proposing conservation strategies. *In situ* conservation will be suitable for *P. micranthum*, because it possess high ability of clonal growth and sufficient genetic diversity[13]. In contrast, an *ex situ* strategy should be taken into consideration for *P. malipoense*, for there left only very few individuals in the field[3]. Since the propagation ability of *C. amoena* was extremely low, artificial pollination may be helpful for the recovery of this species in addition to habitat protection and collection forbidden[14]. Particularly, pollinations between populations should be encouraged because a large proportion of genetic variation resides between populations as indicated above. Similarly, when *ex situ* strategy is needed, the sample should be able to cover all the populations across its distribution. Detailed studies of the reproductive biology, population demography, and ecology of these species are currently under way and should yield valuable information for their conservation.

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三种兰科植物的保护遗传学研究初探

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摘要: 采用随机扩增多态 DNA(RAPD)分析研究了中国 3 种珍稀濒危兰科植物硬叶兜兰(*Paphiopedilum micranthum* Tang et Wang)、麻栗坡兜兰(*P. malipoense* S.C.Chen et Tsi)和独花兰(*Changnienia amoena* Chien)的遗传多样性与群体遗传结构。12 个 RAPD 引物在 2 种兜兰中共扩增出 131 条带。对 4 个硬叶兜兰群体的检测表明其物种水平的多态条带百分率(*PPB*)为 71.6%,Nei 的基因多样度(h)为 0.217 1,Shannon 多样性指数(I)为 0.330 1;4 个群体的平均多样性水平为 $PPB = 45.2\%$, $h = 0.145\ 7$, $I = 0.220\ 4$,低于远交兰花的平均水平。在总遗传变异中,群体间遗传变异占 20.31%,略高于远交物种的平均水平。在物种水平上,麻栗坡兜兰的 *PPB* 为 49.5%, h 为 0.117 4, I 为 0.176 4,均大大低于硬叶兜兰。对 11 个独花兰群体采用 16 个 RAPD 引物共扩增出 119 条带。物种水平 $PPB = 76.5\%$, $h = 0.194\ 1$, $I = 0.305\ 8$ 在群体水平上,上述 3 个指标的平均值则分别为 37.2%、0.119 7 和 0.181 0,均低于远交兰花的平均水平。群体间的遗传变异占 45.27%,遗传分化明显高于远交物种的平均水平。导致 3 个物种遗传多样性偏低而群体间遗传分化较高的主要原因在于人为的过度采挖和生境的片断化。研究结果为兰花保护策略和措施的制定提供了理论基础。

关键词: 兜兰;独花兰;RAPDs;保护遗传学

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