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The extent of clonality and genetic diversity in *Sagittaria lichuanensis* (Alismataceae), an endemic marsh herb in China

Shu-Ying ZHAO[1], Jin-Ming CHEN[2,3,*], Qing-Feng WANG[2,3*], Fan LIU[2,3], You-Hao GUO[1], and Wahiti Robert GITURU[4]

[1]*Laboratory of Plant Systematics and Evolutionary Biology, College of Life Sciences, Wuhan University, Wuhan 430072, Hubei, P.R. China*
[2]*Key Laboratory of Aquatic Botany and Watershed Ecology, The Chinese Academy of Sciences, Wuhan 430074, Hubei, P.R. China*
[3]*Wuhan Botanical Garden, the Chinese Academy of Sciences, Wuhan 430074, Hubei, P.R. China*
[4]*Botany Department, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya*

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ABSTRACT. Genetic variation and clonal diversity of seven natural populations of the rare, highly clonal marsh herb *Sagittaria lichuanensis* were investigated using inter-simple sequence repeat (ISSR) markers. Of the sixty-five ISSR primers screened, seven produced highly reproducible bands. Using these primers, a total of 76 DNA fragments were generated with 22 (29.0%) being polymorphic, indicating lower genetic variation at the species level compared to others in the same genus. With the use of 22 polymorphic markers, we were able to identify 9 genets among the 231 samples analyzed. The proportion of distinguishable genets (*PD*: mean 0.039), Simpson's diversity index (*D*: mean 0.309), and evenness (*E*: mean 0.292) exhibited low levels of clonal diversity compared to other clonal plants. The result implies that sexual reproduction might not have played an important role in these populations. The founder effect or the bottleneck effect could be responsible for the current pattern of the genetic variation revealed in *S. lichuanensis* populations.

Keywords: Clonal diversity; Endemic; Genetic diversity; Marsh herb; *Sagittaria lichuanensis*.

INTRODUCTION

For many plants that propagate vegetatively, the identification of individuals is difficult due to the fact that they can be recognized at two different organizational levels: genets and ramets (Kays and Harper, 1974; Harper, 1977). A genet is a group of genetically identical members of a clone originating from a single zygote whereas a ramet is an independent part of a genet (Cook, 1983). For a clonal plant population, the genetically effective population size cannot be determined from counting the number of ramets present: what appears to be a "large" population may consist of few genotypes (Esselman et al., 1999). Populations consisting of few genets tend to be subject to similar genetic processes that affect any small population, such as inbreeding (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). Thus, determination of the effective population size, identifying the number of genets as well as ramets, is crucial to studying the dynamics and evolution in clonal plant populations (Eriksson, 1993; Escaravage et al., 1998).

It is generally assumed that recruitment from seeds is rare and infrequent in clonal plant species (Eriksson, 1989). A high degree of asexual reproduction is often assumed to be associated with genetic monomorphism (Williams, 1975; Harper, 1977). However, an increasing number of studies have found that clonal populations can be characterized by high genetic diversity (Ellstrand and Rose, 1987; Hamrick and Godt, 1989; Widen et al., 1994; Albert et al., 2003). This genetic diversity may result from initial seedling recruitment (termed ISR by Eriksson, 1993), where the genetic diversity of the founder population has been maintained by vegetative growth, or from repeated seedling recruitment (termed RSR by Eriksson, 1993; Albert et al., 2003).

Sagittaria (Alismataceae) is a worldwide genus that is comprised of approximately 30 species (Chen, 1989). Most of these species combine sexual reproduction with some form of asexual reproduction, such as corms, bulbils, or rhizomes (Chen, 1989). Chen (1989) revised the classification of Chinese *Sagittaria* and clarified eight species (including two new species: *S. lichuanensis*

*Corresponding author: E-mail: jmchen@wbgcas.cn, Tel: +86-27-87617212, Fax: +86-27-87510526 (Jin-Ming CHEN); E-mail: qfwang@wbgcas.cn, Tel: +86-27-87510526, Fax: +86-27-87510526 (Qing-Feng WANG).

and *S. wuyiensis*) and two infraspecific taxa. Wang et al. (2010) treated seven species and two infraspecific taxa of the Chinese *Sagittaria* in the Flora of China. *Sagittaria wuyiensis* is treated as a synonymy of *S. lichuanensis*. In the present study we follow the treatment of Wang et al. (2010). *Sagittaria lichuanensis* is an herbaceous perennial. The species is endemic to China, occurring in paddy fields and marshes in the central, southern, and southeastern regions of the country. In our recent field investigation, only seven natural populations were found in China, one population in Hubei Province, one population in Guizhou Province, two populations in Jiangxi Province, and three populations in Fujian Province. The *S. lichuanensis* populations were relatively small and consequently the species is considered endangered in China (Chen, 1989). The species produces unisexual flowers, and the plants are monoecious. The species can reproduce sexually by selfing or outcrossing, or clonally through bulbils (with 3-17 bulbils in each sheath). However, flowering occurs rarely in the extant populations, and no seedlings have been seen in any natural population site or greenhouse (Zhao S Y, unpublished data). These observations raised the possibility that each population may be clonal. The combination of poor seed production and seedling recruitment, and an inherent capacity for vegetative proliferation, suggested that genetic diversity within each population would likely be low.

In recent years, polymerase chain reaction (PCR)-based molecular markers have been widely used in identifying the different clones in populations of clonal plants (Esselman et al., 1999; Li and Ge, 2001; Albert et al., 2003). The PCR-based DNA markers evolve rapidly enough to be variable within a population and are thus suited to detecting genotypic diversity (Esselman et al., 1999). In the present study we employ inter-simple sequence repeat (ISSR) markers (1) to genetically identify *S. lichuanensis* clones as well as to estimate the diversity within these clones and (2) to estimate the genetic diversity in *S. lichuanensis* populations.

MATERIALS AND METHODS

Plant materials

The plant specimens of *S. lichuanensis* used in this investigation were obtained from all the seven extant populations in China. In each of the seven populations, more than 70% of the individuals were sampled. A total of 231 individuals of *S. lichuanensis* were included in the study. Details on collection sites are given in Table 1. About 5 g of fresh leaves was harvested from each plant and immediately dried in a ziplock plastic bag containing about 70 g of silica gel.

Total DNA extraction and ISSR PCR amplification

Total genomic DNA was isolated from 0.5 g of silica-dried leaf tissue following the procedure described by Chen et al. (2007).

ISSR PCR reactions were carried out in a volume of 25 μl containing 0.25 mM of each dNTP, 2.5μl of 10×Taq buffer [10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$ and 50 mM KCl], 1 mM primer, 1 U Taq Polymerase (Tian Yuan Biotech), and 40 ng of DNA template. Amplification of genomic DNA was made on a PTC-100^{TM} thermocycler (MJ Research, Inc.), and commenced with 4 min at 94°C, followed by 35 cycles of 1 min at 94°C, a 1-min annealing at 55°C and a 2-min extension at 72°C, and a final extension cycle of 7 min at 72°C. Amplification products were resolved electrophoretically on 1.5% (W/V) agarose gels run at 100 V in 0.5×TBE (Tris-boric acid-EDTA), visualized by staining with ethidium bromide, and photographed under ultraviolet light. Sixty-five ISSR primers (SBS Genetech. Co. Ltd., Shanghai, China) were screened on eight randomly selected individuals. The eight samples were amplified twice with the same primer. Seven primers that produced clear and 100% reproducible fragments were selected for further analysis (Table 2).

Data analysis

ISSR bands were scored as present (1) or absent (0) for each sample. The Jaccard coefficient was employed to calculate pairwise band similarities for samples using the program NTSYSpc 2.02 (Rohlf, 1998). A group of samples showing identical band patterns were considered to belong to the same genet. Four measures of genotypic diversity were calculated (Fager, 1972; Ellstrand and Roose, 1987): (1) *G*, the number of genotypes detected; (2) *PD*, the proportion of distinguishable genets, $PD=G/N$; (3) Simpson's index of diversity corrected for finite sample size (Pielou, 1969): $D=1-[\Sigma N_i (N_i-1)/N(N-1)]$, where N_i

Table 1. Locations and sample size (number of individuals) of the seven sampled populations of *Sagittaria lichuanensis*.

Populations	Localities	Coordinates	Sample size
DX-1	Dongxiang, Jiangxi Province	116°33' E /28°06' N	44
DX-2	Dongxiang, Jiangxi Province	116°34' E /28°10' N	29
WY-1	Wuyishan, Fujian Province	117°48' E /27°28' N	41
WY-2	Wuyishan, Fujian Province	117°37' E /27°37' N	12
WY-3	Wuyishan, Fujian Province	117°36' E /27°35' N	20
PB	Pingba, Guizhou Province	106°16' E /26°25' N	39
LC	Lichuan, Hubei Province	108°42' E /30°11' N	48

Table 2. Names and sequences of seven effective ISSR primers used in the present study and the number of bands scored from the primers (Y = C or G).

Primers	Sequences (5'-3')	No. of bands scored	No. of polymorphic bands
SBS808	$(AG)_8C$	12	5
SBS811	$(AC)_8C$	9	3
SBS826	$(GA)_8T$	6	2
SBS827	$(GA)_8C$	13	5
SBS834	$(AT)_8(CG)C$	12	1
SBS835	$(AG)_8(CG)T$	13	2
SBS841	(GA)8YC	11	4

is the number of samples of the *i*th genotype; (4) The genotypic evenness (Fager, 1972), $E=(D-D_{min})/(D_{max}-D_{min})$, where $D_{min}=(G-1)(2N-G)/N(N-1)$ and $D_{max}=(G-1)N/G(N-1)$.

The resulting presence/absence data matrix of the ISSR was analyzed to estimate genetic diversity parameters: the percentage of polymorphic bands (*PPL*), Nei's (1973) gene diversity (*H*) and Shannon's information index (*I*) (Lewontin, 1972). The estimations of genetic diversity were conducted not only at the total species level, but also within populations. All calculations were performed using the POPGENE program, Version 1.31 (Yeh et al., 1997).

RESULTS

Clonal Diversity

We regard ramets with the same ISSR profile as having the same genotype and belonging to the same genet. From the seven ISSR primers, 22 polymorphic markers were generated. A total of 9 different genotypes or clones were identified among the 231 samples analyzed (Table 3). Of these, one genotype was specific to the DX-1 population (clone A), three to WY-1 (clone D, E and F), and two to WY-3 (clone H and I). The DX-1, PB and LC populations share a common clone (clone B), the DX-2 and WY-3 populations share the clone C, and the WY-2, WY-3 and LC populations share the clone G (Table 3).

Based on ISSR data analyses, the proportion of distinguishable genotypes ranged from 0.026 to 0.2 (mean *PD* = 0.072) for *S. lichuanensis* populations. The values of *D* ranged from 0.000 to 0.721 and the values of *E* ranged from 0.000 to 0.865 (mean *D* = 0.208, *E* = 0.283). For the whole species, the values of *D*, *PD* and *E* were 0.309, 0.039 and 0.292, respectively (Table 3).

Genetic Diversity

A total of 76 bands ranging in size from 100 to 1,800 bp were obtained. Of all loci observed in the 231 individuals of *S. lichuanensis*, 29.0% (*PPL*) were polymorphic. Genetic diversity varied among populations with *PPL* values ranging from 0.0% to 23.7% in *S. lichuanensis* populations (Table 4). Shannon's index (*I*) indicated that the WY-1 population had the greatest variation (0.060) while the PB, WY-2, and DX-2 populations showed the least variation (0.000) (Table 4). Nei's gene diversity index (*H*) in this species displayed similar trends to those of the *PPL* values both at population and species level (Table 4).

DISCUSSION

The genotypic diversity values obtained in this study for *S. lichuanensis* are comparable to other clonal plants. Hangelbroek et al. (2002) estimated the expected genotypic diversity parameters (*PD* and *D*) in herbaceous clonal plants to be 0.08-0.94 (mean *PD*: 0.44) and 0.00-1.00 (mean *D*: 0.74), respectively. In the present study the genotypic diversity (mean *PD*: 0.072 and mean *D*: 0.208) detected in *S. lichuanensis* was at the lower end of the estimated values for herbaceous clonal plant species. Furthermore, the levels of genotypic diversity (mean *PD* and *D* values) detected in this study were lower than those estimated for several other species in the family Alismataceae. For example, using RAPD molecular markers, Chen et al. (2006) revealed a high level of clonal

Table 3. Clonal diversity and distribution of clones in populations of *Sagittaria lichuanensis*.

Population	N	G	PD	*D*	*E*	Clones
DX-1	44	2	0.045	0.169	0.266	A (4), B (40)
DX-2	27	1	0.037	0	0	C (27)
WY-1	41	3	0.073	0.227	0.223	D (36), E (2), F (3)
WY-2	12	1	0.083	0	0	G (12)
WY-3	20	4	0.2	0.721	0.865	C (4), G (2), H (9), I (5)
PB	39	1	0.026	0	0	B (39)
LC	48	2	0.042	0.337	0.629	B (38), G (10)
Mean	----	----	0.072	0.208	0.283	----
Species level	231	9	0.039	0.309	0.292	----

**N* = sample size; *G* = number of genotypes; *PD* = proportion of distinguishable genets; *D* = Simpson index; *E* = Fager index; Numbers in parentheses represent number of replications for each clone.

Table 4. Genetic diversity in populations of *Sagittaria lichuanensis*.

Population	No. of polymorphic loci	*PPL* (%)	*H*	*I*
DX-1	3	4.0	0.007 (0.032)	0.012 (0.060)
DX-2	0	0	0.000 (0.000)	0.000 (0.000)
WY-1	18	23.7	0.031 (0.056)	0.060 (0.109)
WY-2	0	0	0.000 (0.000)	0.000 (0.000)
WY-3	5	6.6	0.020 (0.086)	0.031 (0.126)
PB	0	0	0.000 (0.000)	0.000 (0.000)
LC	1	1.3	0.004 (0.038)	0.008 (0.059)
Species level	22	29.0	0.025 (0.078)	0.047 (0.119)

*Numbers in parentheses are standard deviations; *PPL* = Percentage of polymorphic loci; *H* = Nei's gene diversity; *I* = Shannon's information index.

diversity in populations of the rare marsh herb *Caldesia grandis* (mean *PD*: 0.37 and mean *D*: 0.91). Using ISSR molecular markers, Chen et al. (2007) reported levels of clonal diversity in the endangered *S. natans* (mean *PD* = 0.82, mean *D* = 0.95) and its widespread congener *S. trifolia* (mean *PD* = 0.42, mean *D* = 0.89) which were higher than that in *S. lichuanensis*. Using allozyme markers, Chen and Wang (2006) also found a high level of clonal diversity in *S. potamogetifolia*, an endangered and endemic marsh herb in China (mean *PD* = 0.60, mean *D* = 0.96).

The low levels of clonal diversity in *S. lichuanensis* revealed in this study indicate that sexual reproduction may not have played an important role in the establishment or persistence of the *S. lichuanensis* populations. Field investigation showed that flowering did not occur in five out of the seven study populations namely WY-2, LC, PB, DX-1, and DX-2 populations. In the WY-1 and WY-3 populations, we observed several individuals flowering during July and August. Interestingly these populations also maintained the highest level of clonal diversity as revealed by ISSR analysis. This finding indicates that sexual reproduction must have occurred in the history of the populations. Through computer simulation models Soane and Watkinson (1979) and Watkinson and Powell (1993) have demonstrated that the input of only a few seedlings per year may have major effects on amount of clonal diversity within populations. When seedling recruitment continues to take place after establishment of a population, a pattern of many small genets is expected. In contrast, populations with no further seedling recruitment after an initial colonization event exhibit a rapid loss of genets, resulting in a few large genets. In this study, most of the studied populations consist of a few large genets. Hence, the clonal structure in these populations is consistent with the hypothesis that after an initial establishment of seedlings, no further seedling recruitment has occurred in the populations. Conceivably, each population in this study was initially larger in both size and genotypic diversity, possibly having formed during an early colonization episode following large-scale disturbance. The equilibrium level between the selective elimination of genets from the initial colonization event and the increase in the numbers of ramets was determined by the resource availability (Eriksson, 1993). In most of the studied populations, the resource may have been available, and the number of genets might have decreased with time, leading to a gradual dominance of only a few genets. In addition, genet decline may occur as a result of intraspecific competition between genets, or selection against genotypes by local environments (Hartnett and Bazzaz, 1985). However, several small genets were revealed in the structure of WY-1 and WY-3 populations. It seems probable that repeated seedling recruitment occurs in these populations.

An earlier study in genus *Sagittaria* (Chen et al., 2007), comparing the extent of genetic variation in the endangered *S. natans* and its widespread congener *S. trifolia* based on ISSR molecular makers, reported that *S. natans* exhibited a higher degree of genetic variation compared to *S. trifolia*. Recent and on-going decimation of *S. natans* populations in the region appeared not to have had a major impact on genetic diversity in this rare plant (Chen et al., 2007). Unlike the endangered plant *S. natans*, populations of the endangered *S. lichuanensis* exhibited a lower degree of genetic diversity compared to both the widespread *S. trifolia* (*PPL*: 32.6%) and the endangered *S. natans* (*PPL*: 48.9%). This suggests that sexual reproduction is more prevalent in the latter two species than *S. lichuanensis*, with the exception of two study populations, namely WY-1 and WY-3. The presence of flowering individuals and possible occurrence of sexual recombination may have contributed to retention of higher levels of genetic diversity in these populations. An alternative explanation is that *S. natans* and *S. trifolia* might have benefited from the disturbance because it would create physical gaps and allow sexual progeny to recruit successfully among adult genets, resulting in higher genetic diversity (Barrett and Silander Jr, 1992; Kudoh et al., 1999). However, for *S. lichuanensis*, although the

populations might have experienced the same disturbance as *S. natans* and *S. trifolia*, the seeds were sterile (Zhao S Y, personal observation), which might have hindered genetic diversity.

Several previous surveys of genetic variation in plants have shown that rare, endemic, or narrowly distributed plants tend to maintain low degree of genetic variability due to the impact of genetic drift, the founder effect, the bottleneck effect, and directional selection with high levels of inbreeding (Franklin, 1980; Karron, 1987; Hamrick and Godt, 1989; Ellstrand and Elam, 1993). Both the founder effect and the bottleneck effect would suitably explain the pattern of genetic variation revealed in *S. lichuanensis* populations. The effective population size was small, and only nine genets were found in the seven extant populations in this study. These extant genets may represent the initial founders, which were started by a few members of the original population. Due to the absence of further seedling recruitment after an initial colonization event, the colony population may have reduced genetic variation compared to the original population. An alternative explanation is that the current *S. lichuanensis* populations may be remnants of much larger populations which existed earlier and were subject to dramatic natural selection or competition, during which most individuals died without passing on their genes. The few survivors of these evolutionary "bottlenecks" reproduced very successfully afterward via clonal growth, resulting in "large" populations in subsequent generations. The consequence of such a bottleneck effect would be the extraordinary reduction in genetic diversity of this species since most variability is lost at the time of the bottleneck.

Understanding the presence and extent of clonality within and among the remaining populations of the rare *S. lichuanensis* and identifying the true genetic status of this species are crucial first steps to informed decision making for appropriate conservation management. There are very few genets in the remaining *S. lichuanensis* populations, and furthermore they are mostly localized within a single population. Although the clones B, C and G are widely distributed in several populations, each of the genets might harbor different adaptive capacity in different environments. Thus, it is vital to protect and preserve all nine clones across the seven sites, as the loss of any single population may lead to a substantial reduction in the overall genetic diversity remaining within the species. In addition, degradation of their habitats is also a main reason for the rarity of the species; protecting more of these habitats is, therefore, an appropriate conservation strategy. Since reproduction in this species is predominantly vegetative with sexual reproduction being extremely rare or even absent, population manipulations such as hand pollinations are, at present, not an option for introducing more genetic variation into the existing populations, which in some cases totally lack genetic variation. Future research should prioritize determining the reasons that hinder successful sexual reproduction in *S. lichuanensis* with the ultimate aim of developing mechanisms for promoting sexual reproduction in the species and thereby providing materials for increasing genetic diversity.

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LITERATURE CITED

Albert, T., O. Raspe, and A.L. Jacquemart. 2003. Clonal structure in *Vaccinium myrtilius* L. revealed by RAPD and AFLP markers. Int. J. Plant Sci. **164:** 649-655.

Barrett, J.P. and J.A. Jr. Silander. 1992. Seedling recruitment limitation in white clover *Trifolium repens* (Leguminosae). Amer. J. Bot. **79:** 643-649.

Barrett, S.C.H. and J.R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. *In* K.D.A. Fal and K.E. Holsinger (eds.), Genetics and Conservation of Rare Plants. Oxford University Press, New York, pp. 3-30.

Chen, J.H. and X.F. Wang. 2006. Clonal diversity and population differentiation of *Sagittaria potamogetifolia*. J. Huazhong Agri. Univer. **2:** 194-198.

Chen, J.K. 1989. Systematic and Evolutionary Botanical Studies on Chinese *Sagittaria*. Wuhan University Press, Wuhan, China.

Chen, J.M., W.R. Gituru, and Q.F. Wang. 2007. A comparison of the extent of genetic variation in the endangered *Sagittaria natans* and its widespread congener *S. trifolia*. Aquat. Bot. **87:** 1-6.

Chen, J.M., W.R. Gituru, Y.H. Wang, and Q.F. Wang. 2006. The extent of clonality and genetic diversity in the rare *Caldesia grandis* (Alismataceae): comparative results for RAPD and ISSR markers. Aquat. Bot. **84:** 301-307.

Cook, R.E. 1983. Clonal plant population. Amer. Scientist **71:** 244-253.

Ellstrand, N.C. and D.R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Syst. **24:** 217-242.

Ellstrand, N.C. and M.L.Roose. 1987. Patterns of genotypic diversity in clonal plant species. Amer. J. Bot. **74:** 123-131.

Eriksson, O. 1989. Seedling dynamics and life histories in clonal plants. Oikos **55:** 231-238.

Eriksson, O. 1993. Dynamics of genets in clonal plants. Trends Ecol. Evol. **8:** 313-316.

Escaravage, N., S. Questiau, A. Pornon, B. Doche, and P. Taberlet. 1998. Clonal diversity in a *Rhododendron ferrugineum* L. (Ericaceae) population inferred from AFLP markers. Mol. Ecol. **7:** 975-982.

Esselman, E.J., J.Q. Li, D. Crawford, J.L. Winduss, and A.D. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. Mol. Ecol. **8:** 443-451.

Fager, E.W. 1972. Diversity: a sampling study. Amer. Nat. **106:** 293-310.

Franklin, I.R. 1980. Evolutionary changes in small populations. *In* M.E. Soule and B.A. Wilcox (eds.), Conservation Biology. Sinauer, Sunderland, MA, pp. 135-149.

Hamrick, J.L. and M.J.W. Godt. 1989. Allozyme diversity in plant species. *In* A.H.D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir (eds.), Plant Population Genetics, Breeding, and Genetic Resources. Sinauer, Sunderland, pp. 43-63.

Hangelbroek, H.H., N.J. Ouborg, L. Santamaria, and K. Schwenk. 2002. Clonal diversity and structure within a population of the pondweed *Potamogeton pectinatus* foraged by Bewick's swans. Mol. Ecol. **11:** 2137-2150.

Harper, J.L. 1977. Population Biology of Plants. Academic Press, London.

Hartnett, D.C. and F.A. Bazzaz. 1985. The genet and ramet population dynamics of *Solidago canadensis* in an abandoned field. J. Ecol. **73:** 407-413.

Karron, J.D. 1987. A comparison of levels genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. Evol. Ecol. **1:** 47-58.

Kays, S. and J.L. Harp. 1974. The regulation of plant and tiller density in a grass sward. J. Ecol. **62:** 97-105.

Kudoh, H., H. Shibaike, H. Takasu, D.F. Whigham, and S. Kawano. 1999. Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. J. Ecol. **87:** 244-257.

Lewontin, R.C. 1972. The apportionment of human diversity. Evol. Biol. **6:** 381-398.

Li, A. and S. Ge. 2001. Genetic variation and clonal diversity of *Psammochloa villosa* (Poaceae) detected by ISSR markers. Ann. Bot. **87:** 585-590.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA **70:** 3321-3323.

Pielou, E.C. 1969. An Introduction to Mathematical Ecology. Wiley-Interscience, New York.

Rohlf, F.J. 1998. NTSYSpc: Numerical Taxonomy and Multivariate Analysis System, version 2.02. Exeter Software, Setauket, New York.

Soane, I.D. and A.R. Watkinson. 1979. Clonal variation in populations of *Ranunculus repens*. New Phytol. **82:** 557-573.

Wang, Q.F., R.R. Haynes, and C.B. Hellquist. 2010. Alismataceae. Flora of China, Vol. 23 (http://flora.huh.harvard.edu/china/mss/volume23/Flora-of-China-Vol23-Alismataceae).

Watkinson, A.R. and J.C. Powell. 1993. Seedling recruitment and the maintenance of clonal diversity in plant populations-a computer simulation of *Ranunculus repens*. J. Ecol. **81:** 707-717.

Widen, B., N. Cronberg, and M. Widen. 1994. Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. Folia Geobot. Phytotaxon **29:** 245-263.

William, G.C. 1975. Sex and Evolution. Princeton University Press, Princeton, USA.

Yeh, F., R.C. Yang, and T. Boyle.1997. POPGENE. A User-friendly Shareware for Population Genetic Analysis. Molecular and Biotechnology Center, University of Alberta, Edmonton.

中國特有水生草本植物利川慈姑（澤瀉科）的克隆多樣性和遺傳多樣性

趙淑穎[1] 陳進明[2,3] 王青鋒[2,3] 劉 帆[2.3] 郭友好[1] Wahiti Robert GITURU[4]

[1] 中國武漢大學 生命科學學院

[2] 中國科學院 水生植物與流域生態重點實驗室

[3] 中國武漢植物園

[4]Botany Department, Jomo Kenyatta University of Agriculture and Technology
P. O. Box 62000-00200, Nairobi, Kenya

採用 ISSR 分子標記對珍稀瀕危克隆植物利川慈姑現存的 7 個自然居群進行了遺傳多樣性和克隆多樣性研究。通過對 65 個 ISSR 引物的篩選，共選出 7 個具有高度可重複性的引物。利用這 7 個引物對 231 個樣品進行了擴增，共得到 76 條 DNA 帶譜，其中 22 條爲多態性的條帶。該物種在物種水準上具有較低的遺傳多樣性（29.0%）。利用本研究所得到的 22 條多態性的條帶在 231 個樣品中共鑒定出 9 個基因型或基株。可辨別基因型的比例（*PD* 平均值）爲 0.039，Simpson 指數（*D* 平均值）和 Fager 指數（*E* 平均值）分別爲 0.309 和 0.292。相對其它克隆植物來說，利川慈姑具有較低的克隆多樣性。這些結果表明有性生殖在居群遺傳變異中的貢獻較小。而奠基者效應或遺傳瓶頸的作用很可能是導致目前利川慈姑居群遺傳變異模式形成的主要原因。

關鍵詞：克隆多樣性；遺傳多樣性；特有物種；水生草本；利川慈姑。