

Genetic variation and clonal diversity in populations of *Nelumbo nucifera* (Nelumbonaceae) in central China detected by ISSR markers

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Abstract

To obtain accurate estimates of population structure for purposes of conservation planning for wild lotus (*Nelumbo nucifera* Gaertn.) in central China, genetic diversity among and within six populations, and clonal diversity within another two populations of the species were analyzed. The genetic diversity was high (percentage of polymorphic bands, PPB = 90.0%; Shannon's information index, $I = 0.383 \pm 0.234$) at the species level, but low within individual study populations (PPB = 35.8%; Shannon's information index $I = 0.165 \pm 0.241$). The mean coefficient of gene differentiation (G_{st}) was 0.570, indicating that 43.0% of the genetic diversity resided within the population. Analysis of molecular variance (AMOVA) indicated that 50.47% of the genetic diversity among the study populations was attributed to geographical location while 12.3% was attributed to differences in their habitats. An overall value of mean estimated number of gene flow ($N_m = 0.377$) indicated that there was limited gene flow among the sampled populations. The level of clonal diversity found within the populations was considerably high (Simpson's diversity index, $D = 0.985$) indicating that clonal diversity contributes to a major extent to the overall genetic variation in the genetic structure of *N. nucifera*. On the basis of the high G_{st} and D values detected in this study we recommend that any future conservation plans for this species should be specifically designed to include those representative populations with the highest genetic variation for both *in situ* conservation and germplasm collection expeditions.

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1. Introduction

Over the long term, the ability of a population to respond adaptively to environmental changes depends on the level of genetic variability or diversity it contains (Ayala and Kiger, 1984). During the process of evolution, genetic differentiation by natural selection to facilitate reproductive isolation involves the presupposition of the origin of geographic races, subspecies, and species (Stebbins, 1999). A species without enough amount of genetic diversity is thought to be unable to

cope with changing environments or evolving competitors and parasites (Schaal et al., 1991). Therefore, investigation of population genetic diversity and the structure of populations within a species may not only illustrate the evolutionary process and mechanism, but also provide information useful for biological conservation (Schaal et al., 1991). Clonal plants present special problems when analyzing populations because a single genetic individual (genet) may comprise numerous morphological units (ramets) that appear distinct (Parks and Werth, 1993). Therefore, it is difficult to detect population dynamics because the number of genets in a population is unknown. Furthermore estimates of individuals based on population size are usually subject to error. Regardless of ramet numbers, populations of clonal plants consisting of few genets tend to be subject to similar genetic processes that affect any small population, such as genetic drift and inbreeding (Sipes and Wolf, 1997). Thus, for clonal species, genet identification is

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usually a necessary precursor to population genetic studies (Li and Ge, 2001).

The Nelumbonaceae also known as the lotus family is a small family of perennial, aquatic angiosperms which consists of the two species *Nelumbo nucifera* Gaertn. and *Nelumbo lutea* (Willd.) Pers. The family is characterized by simple, peltate leaves which lack stipules and are borne on the surface of the water. *N. nucifera*, also called the Indian or sacred lotus, is found throughout Asia and Australia, whereas *N. lutea*, also known as the American lotus or water chinquapin, occurs in eastern and southern North America. *N. nucifera* is an important aquatic economic plant, not only as a dainty and ornamental flower but also as a source of herbal medicine with strong antipyretic, cooling, astringent, and demulcent properties (Mukherjee et al., 1997; Sinha et al., 2000; Qian, 2002). The species is of religious significance in SE Asia (hence, the name sacred lotus) and the seeds and leaves are also eaten in this region. Wild lotus populations are an important resource for breeding of cultivated lotus.

The insect-pollinated, aquatic, herbaceous macrophyte, *N. nucifera* may reproduce by sexual (seeds) and asexual (rhizomes) means. In recent years, however, local populations of wild lotus in central Mainland, China, have been greatly reduced due to the rapid development of the aquaculture industry. *N. nucifera* has been included in the list of endangered species in China (Dong and Zheng, 2005). In dwindling populations the size of the surviving population greatly affects genetic diversity (Gao, 2005). Therefore, arresting the decline of populations of *N. nucifera* in the wild and studying the structure of the remaining populations, especially the extent of genetic diversity still retained in them, is of critical importance. Up to the present, no report has been made on the genetic diversity, clonal diversity, and population structure in populations of *N. nucifera* in central Mainland, China. Our study was intended as a step towards ending this dearth of information on this important natural resource.

Inter-simple sequence repeat (ISSR) is a technique that uses repeat-anchored primers to amplify DNA sequences between two-inverted SSR (Zietkiewicz et al., 1994). ISSR markers do not require a prior knowledge of the SSR target sequence, furthermore, they are highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature. ISSR markers have been found to provide highly polymorphic fingerprints (Zietkiewicz et al., 1994; Kojima et al., 1998; Bernet et al., 2002). Recent ISSR studies of natural

populations have demonstrated the hypervariable nature of these markers and their potential use for population level studies (Chen et al., 2005, 2006). The genetic diversity of several aquatic plant species including clonal aquatics have been successfully assayed using ISSR markers (Chen et al., 2006).

In this study, we investigated the genetic diversity and clonal diversity in the diminishing wild populations of *N. nucifera* in central Mainland, China, with the aim of providing insights into their population genetics to facilitate conservation management of the remaining populations. Appropriate conservation management should be adopted including *in situ* conservation and germplasm collection from those of the remaining populations with the greatest genetic variation.

2. Materials and methods

2.1. Plant materials

N. nucifera is a perennial aquatic plant which produces insect-pollinated flowers with copious quantities of pollen. In the Yangtze River drainage area the plant flowers from April to September. *N. nucifera* prefers calm adlittoral habitats such as lakes, swamps, pools and rice fields. A single individual may occupy an area of 2–4 m² while the main rhizome may reach 3–6 m (Wang and Zhang, 2005). Dispersal may occur during the sporophyte phase by means of water- and bird-dispersed fruits and vegetative fragments, as well as during the gametophyte phase by means of insect-dispersed pollen.

The plant material used in the genetic diversity study was obtained from six populations, two each from three lakes in the middle reaches of the Yangtze River basin in central Mainland, China (Table 1). The intervals between samples (10 m for genetic diversity studies and 3 m for clonal diversity studies) employed during sampling were chosen in consideration of the extended length of the main rhizome in *N. nucifera* and after preliminary observations revealed the expansive area occupied by a single individual in the study populations. For analysis of genetic diversity, several representative samples of 15 plants each were obtained from each of the six study populations at intervals of 10 m apart. About 5 g of young leaves from each individual plant (the term individual is used here to represent a sampling unit such as a physiologically distinct plant unit (clonal or non-clonal) at a certain distance from each other and does not necessarily

Table 1
Location, sample size, and statistical analysis of genetic variation in the populations of *Nelumbo nucifera* (PPB: the percentage of polymorphic band; *I*: Shannon's information index)

Population	Location	Sample size	PPB (%)	<i>I</i>
Baoan A (BAa)	Baoan Lake, 114°36.062', 30°16.673'	15	28.1	0.115 (0.205)
Baoan B (BAb)	Baoan Lake, 114°42.678', 30°21.990'	15	36.0	0.16 (0.244)
Liangzi A (LZA)	Liangzi Lake, 114°31.506', 30°09.629'	15	35.4	0.160 (0.242)
Liangzi B (LZb)	Liangzi Lake, 114°16.813', 30°16.862'	15	49.4	0.252 (0.279)
Niushang A (NSa)	Niushang Lake, 114°22.715', 30°22.032'	15	30.5	0.141 (0.234)
Niushang B (NSb)	Niushang Lake, 114°21.161', 30°22.721'	14	35.4	0.156 (0.234)
Species level		89	90.2	0.383 (0.240)

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