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Short communication

Clonal diversity and structure of the invasive aquatic plant *Eichhornia crassipes* in China

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Abstract

The information on diversity and spatial distribution of clones of an invasive clonal plant is crucial for the understanding of its clonal structure and invasive history. In this paper, random amplified polymorphic DNA (RAPD) markers were used to explore the clonal diversity and clonal structure of *Eichhornia crassipes* (Mart.) Solms in natural populations, and their possible effects on the plant success as an invader are also discussed. Five populations covering the entire distribution area in China were studied, sampling 43 individuals per population at an interval of 1 m in a sampling plot. Twelve RAPD primers produced 69 reproducible bands, with 22 being polymorphic. Only five RAPD phenotypes (clones) were detected in these five populations, but each population consisted of at least three clones, contrary to the traditional expectations that *E. crassipes* populations should be monoclonal. The diversity of clones within populations is thought to be mainly resulted from multiple introductions by humans. The evenness of distribution of clones varied slightly and most clones were widespread, suggesting clonal growth is the predominant mode of regeneration in all the populations. A single clone dominated each population and this clone might be the first one introduced into China or the genotype with a higher phenotypic plasticity, which could survive and reproduce via clonal growth in various habitats. The clones in each population were highly intermixed, especially in river populations, suggesting this species has a guerilla clonal structure which can be facilitated by water current.
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Keywords: *Eichhornia crassipes*; Biological invasion; Clonal diversity; Clonal structure; Random amplified polymorphic DNA (RAPD)

1. Introduction

For clonal plants, reliable information on population dynamics and detailed demographic data are usually difficult to obtain without genetic analyses of population (Sipes and Wolfe, 1997; Ge et al., 1999). For example, one cannot know whether a population of ramets consists of one or many clones (clonal diversity), and how the individuals of different clones are spatially distributed (i.e. clonal structure, which is determined mainly by the patterns of clonal growth and clonal diversity). Therefore, clonal diversity and clonal structure have long been important topics in studies of plant ecology (Sipes and Wolfe, 1997; Fischer et al., 2000). It is believed that clonal diversity and clonal structure can significantly increase plant fitness and buffering against environmental heterogeneity (Eckert, 1999; Eckert et al., 2003). For the invasive clonal plants, the information on clonal diversity and structure may be of great importance for detecting their demographic dynamics and invasive history. For example, clonal plants often show two kinds of clonal structure, phalanx versus guerilla (Lovett Doust, 1981). The phalanx type of clonal structure can greatly enhance plant's competitive ability via close clusters of ramets, while the guerilla type is normally adapted for food foraging and plant dispersal (Lovett Doust, 1981; Sutherland and Stillman, 1988; Chung et al., 2000), which could greatly enhance the population spread and sometimes cause an invasion. Furthermore, the invasive history of an invasive plant could be seen from genotypic diversity (a high genotypic diversity normally indicated multiple invasions and vice versa) and the genetic relationships of invaded populations. Unfortunately, for many invasive plants, the patterns of clonal structure in their natural populations are still in lack of studies. In many cases, the lack of

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detailed information on the invader's clonal diversity and clonal structure made it difficult to understand the population dynamics and invasive history.

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms, Pontederiaceae) is a clonal, aquatic weed with a cosmopolitan distribution. It forms dense mats that interfere with navigation, recreation, irrigation, and power generation in tropic and subtropic regions. This plant's invasive ability results mainly from extensive clonal growth by stolons and free-floating life forms (Barrett, 1992), so that the number of ramets can double in less than 1 week (Holm et al., 1977), and allows an easy dispersal via water current. This plant has a tristylous breeding system, which consists of three floral morphs differing in lengths of style and stamens to promote outcrossing among morphs (Barrett, 1980a,b). Most *E. crassipes* populations in the invaded regions consist only of mid-styled flowers (M morph), sometimes with another morph (long styled) (Barrett, 1980a,b; Ren et al., 2005). Despite the large body of scientific literature about this species including physiological characteristics and the pattern of clonal growth in the greenhouse (Watson and Cook, 1982; Geber et al., 1992), sexual reproduction (Barrett, 1980a,b), and genetic variation (Ren et al., 2005; Li et al., 2006), clonal diversity and clonal structure in natural populations are not well known.

Here, we report our research on the clonal diversity and structure of natural *E. crassipes* populations throughout China by random amplified polymorphic DNA (RAPD) fingerprinting. As previous genetic studies (Ren et al., 2005; Li et al., 2006) using the same RAPDs method showed that this species has very low genetic variation across a large geographic region in China, we chose five representative populations covering its entire Chinese distribution. The main objective of this study was to analyse the number and size of clones (clonal diversity) and how they are spatially distributed (clonal structure) in natural populations. Our particular interest was to investigate the possible variations of clonal diversity and structure in different populations and how the population history affect them, which could provide some useful insights into the clonal structure and invasive history of this "worst" weed (Holm et al., 1977; Barrett, 1992).

2. Materials and methods

2.1. Study sites and sampling method

In China, *E. crassipes* is generally found in small lakes and rivers near big cities in the southern region. In August and September of 2003, five cities covering the entire distribution area of the species in China, Wuhan (Central China. Hereafter WH), Shanghai (East China. SH), Fuzhou (Southeast China. FZ), Kunming (West China. KM), and Hainkou (on Hainan Island in South China. HK) were selected as sampling localities (Table 1). In each locality, one population of *E. crassipes* with near 100% cover of the water surface was selected as the sampling population. The distances between these populations were 730 km (Wuhan–Shanghai), 591 km (Shanghai–Fuzhou), 1186 km (Fuzhou–Haikou), 1002 km (Haikou–Kunming), and 1391 km (Kunming–Wuhan).

In each population, plants were collected in a rectangular sampling plot of 10 m × 2 m selected from a homogeneous part of the population. Thirty-three fresh leaves (one per individual) of *E. crassipes* were collected at each point of intersection of a 1 m × 1 m grid. Along the lengthways of the sampling plot, five additional individuals at both sides were collected at an interval of 1 m to get more detailed information. Therefore 43 individuals in each population were sampled altogether, except Population SH, where 6 plants were missing because the sites were occupied by stones or other plants). The exact geographical location of sampled individuals was recorded, and harvested leaves were preserved in silica gel until DNA extraction. DNA was isolated according to the protocol of Doyle and Doyle (1987). DNA quality and quantity were determined visually by comparing with DNA marker DL2000 (TaKaRa, Japan) on 0.8% agarose gels.

2.2. RAPD-PCR amplification

The same primers for the analysis of genetic variation on *E. crassipes* described in Ren et al. (2005) were also used in this study (Table 2). DNA amplification was carried out in a PTC-100 thermal cycler (MJ Research). Reactions were conducted in a volume of 10 μL containing 50 mM Tris–HCl (pH 8.0), 500 mg/mL KCl, 2 mM $MgCl_2$, 200 μM dNTP, 1 μM primer, 5 ng of DNA template, and 0.4 U Taq polymerase (Huamei Company, China). The PCR programme involved pre-denaturation at 94 °C for 4 min, followed by 40 cycles of 20 s at 94 °C, 1 min at 36 °C, 2 min at 72 °C, and ended with 7 min at 72 °C. Samples were held at 4 °C until next step. A negative tube was included in each amplification run to check for contamination. Each PCR experiment was carried out at least twice to ensure consistency and reproducibility. Amplification products were resolved electrophoretically on 1.5% agarose gels run at 200 V in 1× TBE on DYY-III produced by Liuyi Factory, China. The bands were visualized by staining with ethidium bromide, and photographed under ultraviolet light. The sizes

Table 1
Locations and habitats of populations sampled

Population	Geographic location	Population size (no. of ramets)	Habitat	Sampling site	Growth status (floating:rooted)	Floral morph
Wuhan (WH)	32°37′N; 114°11′E	≈5,000	Lake	Near shore	0.9:0.1	M
Shanghai (SH)	31°10′N; 121°18′E	≈600	Stream	Near shore	1:0	M
Fuzhou (FZ)	26°01′N; 119°23′E	≈4,000	Small lake	Near shore	1:0	M
Kunming (KM)	25°01′N; 102°38′E	≈800	Pond	Near shore	1:0	M
Haikou (HK)	19°59′N; 110°21′E	≈10,000	Lakefront	On shore	0:1	M

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