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Genetic variation in populations of the threatened seagrass *Halophila beccarii* (Hydrocharitaceae)

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ABSTRACT

Halophila beccarii (Hydrocharitaceae), a small monoecious seagrass, has been listed as a threatened species. In this study, a total of 106 samples were collected from four Chinese populations located at the northern limit of its distribution range. Using six polymorphic microsatellites, we found low genetic variation in this species, in which the mean number of alleles per locus was 2.8, and 16 multi-locus genotypes were revealed. In the four populations, the mean number of alleles per locus ranged from 1.2 to 2, one to eight genotypes were found, and clonal diversity ranged from 0 to 0.23; observed and expected heterozygosity ranged from 0.17 to 0.40 and from 0.09 to 0.24, respectively. Strong genetic differentiation was found among the populations, and the standardized fixation index (F'_{ST}) was 0.787. Species traits (i.e., clonal growth) and bottleneck effects due to drastic population fluctuation may contribute to the observed low genetic variation.

1. Introduction

Theoretical and empirical studies have confirmed that genetic variation is the basis for populations adapting to changing environments (Frankham, 2005; Zhao et al., 2006). High genetic diversity can promote the performance of a population due to selection effects, i.e., having a high probability to contain advantageous genotypes (Hughes and Stachowicz, 2004), or non-additive interactions, such as facilitation, complementary effects (Wang et al., 2012). Populations of threatened species, in general, are of small sizes and high degree of isolation between populations (Lu et al., 2006). Thus genetic drift will play a critical role in shaping genetic structure, and lead to a loss of alleles and a decrease in heterozygosity (Ouborg et al., 2006). Decreased genetic variation may have instantaneous effects, e.g. inbreeding depression and reduced fitness, and long-term negative consequences, i.e., decreased potential to adapt to changing environments. Therefore, extinction risk is expected to be high in the threatened species (Spielman et al., 2004).

Halophila (Hydrocharitaceae), a group of paddle-bladed marine monocotyledons, is the largest genus of seagrasses (Short et al., 2011), although there has been debate on the delimitation of the species in this genus (Short et al., 2010). About 17 *Halophila* species have been reported across five of the six bioregions of the world (Short et al., 2011). *Halophila* species are the most widely distributed tropical seagrasses, and occur mainly in the tropical Indo-Pacific and tropical Atlantic (Short et al., 2007a). The tropical Indo-Pacific has the most abundant seagrasses. With another two newly described species (Kuo et al.,

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2006; Kuo, 2007), a total of 12 *Halophila* species have been reported in this bioregion (Short et al., 2007a). China is the northern distribution limit of tropical seagrasses, and has four *Halophila* species: *Halophila beccarii*, *Halophila decipiens*, *Halophila minor* and *Halophila ovalis* (Yang et al., 2002; Yip and Lai, 2006; Shi et al., 2010).

Halophila beccarii Aschers., the sole species of section *Microhalophila*, is a small monoecious seagrass. This species is characterized by distinct erect lateral shoots, which bear a pseudo-whorl of 4–10 sheathing petiolate leaves (den Hartog and Kuo, 2006). *Halophila beccarii* is disjointedly and fragmentedly distributed in the Indo-Pacific (Short et al., 2007b). The abundance of fruit is low in this species, and the only study carried out in Malaysia found that fruit density was 11.0 ± 1.5 m^{-2}, with a mean of 1.7 seeds per fruit (Zakaria et al., 1999). *Halophia beccarii* has experienced a rapid decline in most distribution sites due to natural and anthropogenic disturbances, and is listed as one of the few threatened seagrass species (Short et al., 2007b, 2011). Similar to other *Halophila* species (Xu et al., 2011), *H. beccarii* is a clonal species, with a rapid rhizome extension rate, and can colonize some areas within a short period of time. However, it can also disappear quickly when habitats become less favourable (Yip and Lai, 2006). Drastic changes in habitat sizes can lead to fluctuations in population size, resulting in negative genetic consequences, such as reduced genetic diversity and enhanced inbreeding and population divergence, which in turn affect population growth and viability, especially under the effects of global warming and anthropogenic disturbances. However, information on this species was scarce.

Seagrass species may maintain relatively low genetic diversity, which was frequently observed using molecular marker allozymes, random amplification of polymorphic DNA, and restriction fragment length polymorphism (Reusch, 2001). However, after Arnaud-Haond et al. (2005) compared the genetic diversity in the seagrass *Posidonia oceanica* using different markers, they proposed that the low genetic variation in clonal plants could be attributed to a low resolution of markers, and suggested that high polymorphic markers, such as microsatellites, should be used in such analyses.

There were four extant *H. beccarii* populations in mainland China, and the largest one was only about 6 ha (Table 1). In view of the very small population size and strong clonal growth in this species, we predicted a low genetic diversity in this threatened seagrass species. In order to test this hypothesis, we collected samples from these populations and genotyped them using polymorphic microsatellite loci developed in this species.

2. Materials and methods

2.1. Sample collection and genotyping

Based on previous surveys (unpublished data) and reports of the distribution of *H. beccarii* (Yip and Lai, 2006; Shi et al., 2010; Huang et al., 2010), we performed an extensive survey from the winter of 2008 to the summer of 2011, and found only four remaining populations. We collected samples from the four extant populations found in mainland China (Table 1, Fig. 1). Geographic coordinates of the four populations were recorded using an eTrex H GPS (UniStrong, Inc., Beijing, China). As seagrass beds are small (<1 ha ∼ about 6 ha), we collected 10-38 shoots randomly at a distance of >2 or 5 m between samples (Table 1). Leaf materials were washed in fresh water, cleaned with absorbent paper, and dried with silica gel.

The genomic DNA was extracted from about 0.03 g dried leaf or root tissues using Plant Genomic DNA kit (Tiangen, Beijing, China). Each sample was genotyped using six microsatellite markers (HB545, HB569, Hb659, HB673, HB870, and HB884) developed in a previous study (Jiang et al., 2011). Forward primers of these 6 primer pairs were labelled with fluorescent dye HEX (HB545, HB569, and HB659), ROX (HB870 and HB884) or 6-FAM (HB673). The protocols of polymerase chain reaction were: 5 min denaturation at 94 °C; 35 cycles of 30 s at 94 °C, 45 s at 53–66 °C, 45 s at 72 °C, and a final extension at 72 °C. PCR products were scanned by an ABI 3130 automated sequencer (Applied Biosystem, Foster City, CA, USA) using an internal lane standard (GS500(-250)LIZ).

2.2. Data analyses

Observed identical multilocus genotypes (MLGs) can either be the result of sampling the same genet, or two different genotypes originated from two distinct sexual reproduction events but sharing the same MLG. In order to address the issue,

Table 1
Parameters of clonal structure and genetic composition for four *Halophila beccarii* populations from coastal regions of South China.

Location	Abbr.	Geographic coordinates	Seagrass bed	Area (ha)	N[a]	DS (m)	Clonal diversity					Genetic diversity			
							G	n	R	$D*$	$ED*$	H_O	H_E	A	F_{IS}
Upper baini, Hong Kong	BN	N22°26′23.8″, E113°56′50.2″	Monospecific	<1	27	2	5	27	0.15	0.62	0.62	0.40	0.24	1.67	−0.69
Donghai Island, Guangdong	DH	N21°06′31.7″, E110°18′45.9″	Monospecific	5	38	5	5	32	0.13	0.37	0.28	0.17	0.24	2.00	0.29
Liushabay, Guangdong	LS	N20°26′6.0″, E109°57′6.0″	Mixed with *Halophila minor*, *Haludule pinifolia*	6	31	5	8	31	0.23	0.77	0.74	0.19	0.24	1.67	0.18
Ronggenshan, Guangxi	GS	N21°29′45.78″, E109°41′14.1″	Mixed with *Zostera japonica*	<1	10	2	1	10	0	0	–	0.17	0.09	1.17	−1.00

[a] Abbreviations: N, number of samples; DS, minimum interval between samples; G, number of genotypes; n, numbers of clones; R, (G-1)/(N-1); D^*, Simpson diversity complementary index; ED^*, Simpson diversity uniformity complementary index; H_O, observed heterozygosity; H_E, unbiased heterozygosity; A, number of average alleles; F_{IS}, inbreeding coefficient.