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ORIGINAL PAPER

Invasion genetics of *Chromolaena odorata* (Asteraceae): extremely low diversity across Asia

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Abstract *Chromolaena odorata* is a native of America while a weed in many parts of tropical and subtropical regions in the world. Research into the invasion mechanisms of *C. odorata* contributes to a broader understanding of factors that facilitate plant adaptation, and also helps developing effective management strategies. In this study, we used three DNA fragments and six microsatellite loci: (1) to compare genetic diversity of *C. odorata* in its native and invaded regions; (2) to elucidate the invasive routes and identify possible source locations of *C. odorata* from America to Asia, with attempt to evaluate the possible mechanisms facilitating the successful invasion of this species. Despite two recorded independent introductions, DNA sequence data revealed only one single haplotype of *C. odorata* present throughout tropical Asia. All six microsatellite loci consistently exhibited extremely low genetic diversity in Asian populations compared to those from native ranges. Our results implied that there was likely only a single introduction to Asia, and Trinidad, Tobago and adjacent areas in the West Indies were the most likely source location of that introduction. The successful invasion of *C. odorata* in Asia may have been facilitated by the genotype with strong competitive ability.

Keywords *Chromolaena odorata* · Genetic diversity · Haplotype network · Invasion genetics · Phylogeography · Source location

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Introduction

The study of evolutionary changes in genetic architecture and introduction history of invasive species is of significance in understanding the ecological and evolutionary factors underlying successful invasions (Sakai et al. 2001; Roman and Darling 2007). Understanding to the evolution of invasiveness in plant species is also central to the success of management

efforts, particularly in the face of anthropogenic redistribution of species at global scale, and predicted increases in species motility due to climate change (Dukes and Mooney 1999; Stachowicz et al. 2002; Müller-Schärer et al. 2004). Molecular methods and associated statistical analyses provide great opportunities to explore the ecological and evolutionary issues regarding biological invasions (Ward and Jasieniuk 2009). Molecular approaches are particularly powerful in tracking geographical origin and introduction histories of invasive species, and in assessing the role of genetic variation and natural selection in invasion success (Estoup and Guillemaud 2010; Perdereau et al. 2013).

Genetic variation plays an important role in the success of invasion in the stages of establishment and range expansion (Sakai et al. 2001). Although it has been suggested that genotypic diversity could enhance the invasive ability (e.g. Wang et al. 2012), invasive species are capable of achieving prosperous colonization (Prentis et al. 2008) regardless of the risk of extinction due to low genetic diversity (Frankham and Ralls 1998). Founder effect can occur when a potentially invasive species is introduced into a new environment (Novak and Mack 2005; Hawley et al. 2006). Consequently genetic diversity is reduced and such a decrease in genetic diversity has even been presumed to be a feature of invasive species (Dlugosch and Parker 2008). Some invasive species can be successful only because of the low genetic diversity. For example, low genetic diversity reduces intraspecific (inter-nest) aggression in ants (Tsutsui 2000). Several researchers have proposed that invasive genotypes characterized by better adaptive ability might facilitate successful invasion (Fuentes-Contreras et al. 2004; Le Roux et al. 2007; Zepeda-Paulo et al. 2010; Harrison and Mondor 2011). Such genotypes may be novel and were generated through recombination resulting from multiple introductions or hybridization (Ellstrand and Schierenbeck 2000; Lavergne and Molofsky 2007), or genotypes with stronger competitive ability, i. e. more invasive than others (Allendorf and Lundquist 2003; Zhang et al. 2010). On the other hand, some studies have also shown that some invasive plants are often genetically diverse (Bossdorf et al. 2005), partially because multiple introductions are common (Genton et al. 2005; Kirk et al. 2011). Different lineages sometimes colonize geographically proximate locations leading to opportunities for admixture, and genetic diversity may be further elevated as a result of recombination.

Chromolaena odorata (L.) R. M. King and H. Robinson (Asteraceae), native to the Americas from southern USA to northern Argentina, is a major threat to diversity and function in a wide variety of ecosystems, ranging from tropical rain forests to savannas in many humid tropical and subtropical biodiversity hotspots of Oceania, Micronesia, Africa and Asia (McFadyen and Skarratt 1996; Kriticos et al. 2005; Raimundo et al. 2007). *C. odorata* was supposed to be apomictic autohexaploid ($2n = 60$) originated from triploid *C. squalida* (DC.) R. M. King and H. Rob. (Coleman 1989). Though it is mainly reproduced by seeds (Coleman 1989), *C. odorata* is also reported to be capable of clonal propogation (Gautier 1993; Liu et al. 2006 and our filed observation). This species is pollinated by insects; and the small and light seed is principally dispersed by wind (Ghazoul 2004; Lakshmi et al. 2011). *C. odorata* has caused serious problems in plantations of perennial crops, pastures and vacant land in regions that have been invaded (De Rouw 1991; Muniappan et al. 2005) and has been listed as one of the world's 100 worst invasive weeds (Lowe et al. 2000). Historical records have indicated that a number of introductions of *C. odorata* have occurred in Asia and invasion of *C. odorata* across Asia was possibly initiated from at least two different source locations (Muniappan et al. 2005). *C. odorata* was first introduced to the Calcutta Botanic Garden in India as an ornamental plant in 1845 from where the species escaped to many other localities in Asia (McFadyen 1989; Muniappan et al. 2005). Jamaica was considered as the possible geographic source of *C. odorata* to India, as at that time, Calcutta and Kingston (Jamaica) was the capital of British India and the administrative center for the British West Indies, respectively. Officials within the British Colonial Service, such as the government botanist or government medical officer were regularly transferred between India and Jamaica (McFadyen 1993). This species was again accidentally introduced, possibly from the West Indies, in the ballasts of cargo boats into Singapore and Malaysia around 1920 (Biswas 1934; Bennette and Rao 1968). *C. odorata* was recorded in northern Australia around 1994 (Waterhouse 1994), notably as two morphological forms. Scott et al. (1998) sequenced the ITS1 (internal transcribed spacer) fragment of 11 samples from native regions

representing three countries (USA, Colombia and Brazil), 12 samples from Australia, seven from Java (Indonesia) and one from Thailand. Those authors found that the more widespread form from Australia genetically matched the Asian biotype, whereas the more localized form matched the biotype from southern Brazil that was accordingly proposed as one source location.

Previous researches have been conducted on the genetic diversity of *C. odorata*, but some important questions remain unresolved. Ye et al. (2004) reported low genetic variation in *C. odorata* in southern China (27 populations) using inter-simple sequence repeats (ISSRs). Recently, a study applying the same molecuar markers (ISSRs) revealed that *C. odorata* in Southern Africa originated from Jamaica and Cuba, and the samples from Asia showed an affinity with samples from Trinidad, Florida and Venezuela (Paterson and Zachariades 2013). It is not clear whether low genetic diversity is a common feature of *C. odorata* across Asia where the species has a history of introduction of over 100 years. It is also uncertain as to whether hybridization occurred between genotypes from, presumably, different native sources of *C. odorata* during this long history of invasion in Asia. Finally, there remains a lack of clarity regarding the specific native locations and original genotypes of the species that were introduced to Asia. In this study we used both maternally inherited chloroplast DNA (cpDNA) and biparentally inherited nuclear DNA (ITS and microsatellite loci) to investigate population genetic architecture and the phylogeographic haplotype network in *C. odorata* sampled across its range in Asia. We aimed to determine whether *C. odorata* exists as a single invasive genotype in Asia, or as multiple lineages given the documented initiation of invasion from more than one site in Asia. We then attempted to trace the invasive routes and identify possible source locations of the invasion of *C. odorata* into Asia. Last, we discussed the possible mechanisms that may have promoted the successful invasion of *C. odorata* into Asia.

Materials and methods

Material collection and DNA extraction

Sampling in the native range of *C. odorata* spanned latitudes from 10°N to 27°N America and included 155 individuals from 17 locations (Table 1; Fig. 1a). In Asia (invaded region), 367 individuals from 29 locations were sampled from ten countries across tropical and subtropical Asia (8°S–25°N) (Table 1, Fig. 1a). Healthy leaves of *C. odorata* were harvested and stored in zip-locked bags containing activated silica gel before further processing. Total genomic DNA was extracted from the leaf tissues following a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987) as described in Yu and Li (2011). All leaf materials and DNA samples were deposited in Laboratory of Plant Phylogenetics and Conservation at Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

DNA sequencing

Since low genetic diversity of *C. odorata* in China has been reported previously (Ye et al. 2004) and from our preliminary study, we sequenced two individuals per population sampled from China for phylogeographic analysis. Three to ten samples per population from other countries in Asia and native regions were sequenced. In total, 129 individuals from 17 populations in the native range of the species and 118 individuals from 29 populations in the invaded regions (Asia) were sequenced (Table 1).

Chloroplast DNA (cpDNA) fragments (*atp*B-*rbc*L and *psb*A-*trn*H) and nuclear internal transcribed spacers (ITS) were amplified using the primers shown in Table 2. Polymerase chain reaction (PCR) amplifications were performed using an ABI Gene Amp 9700^{TM} PCR system in a volume of 25 μL containing 2.5 μL of 10× buffer, 0.2 mM of each dNTP, 2.0 mM of $MgCl_2$, 0.4 μM of each primer, 1U of Taq polymerase (Takara) and 20 ng of genomic DNA. The amplification conditions included an initial denaturing at 94 °C for 3 min, followed by 35 cycles of 40 s at 94 °C, 45 s at the annealing temperature (Table 2) and 45 s at 72 °C, and then a final extension for 10 min at 72 °C. PCR products of two chloroplast fragments were directly sequenced on an ABI 3730 DNA Sequence Analyser (Applied Biosystems, Foster City, California, USA). For ITS, the PCR products were separated by excision and elution from Agarose gel using an Omega Gel Extraction Kit (Omega) before sequencing. We used standard nucleotide ambiguity codes to identify heterozygous sites

Table 1 Distribution of sampling sites of *Chromolaena odorata* and outgroup *Ageratina adenophora*

ID	Location	Longitude	Latitude	Altitude (m)	N_{DNA}	N_{SSR}	Specimen accessions
Asia							
ML	Yunnan, China	101°16′E	21°55′N	577	2	14	HITBC 135950
BB	Yunnan, China	101°35′E	21°35′N	677	2	5	PPC
YW	Yunnan, China	101°28′E	21°58′N	1,312	2	5	PPC
MH	Yunnan, China	101°41′E	21°11′N	859	2	6	PPC
LC	Yunnan, China	99°55′E	22°33′N	1,018	2	10	PPC
JD	Yunnan, China	100°50′E	24°26′N	1,877	2	15	PPC
SM	Yunnan, China	100°56′E	22°46′N	1,380	2	5	PPC
HK	Yunnan, China	103°55′E	22°40′N	320	2	15	PPC
MK	Yunnan, China	98°52′E	25°26′N	838	2	5	PPC
YG	Yunnan, China	99°14′E	24°34′N	1,827	2	5	PPC
YJ	Yunnan, China	97°34′E	24°43′N	252	2	15	PPC
SY	Hainan, China	109°12′E	18°19′N	23	2	10	PPC
QZ	Hainan, China	109°50′E	19°02′N	358	2	15	PPC
GZ	Guangdong, China	113°15′E	23°15′N	5	2	17	PPC
FCG	Guangxi, China	107°58′E	22°08′N	250	2	30	PPC
NN	Guangxi, China	108°21′E	22°49′N	85	2	20	PPC
BS	Guangxi, China	106°38′E	23°53′N	140	2	10	PPC
WX	Vientiane, Laos	102°36′E	17°57′N	170	3	10	PPC
PH	Phongsali, Laos	102°06′ E	21°41′ N	1,350	7	20	PPC
YN	Nshe An, Vietnam	104°53′E	19°03′N	55	10	10	PPC
TL	Chiang Rai, Thailand	99°21′E	19°19′N	530	10	30	PPC
BL	Bangalore, India	77°35′E	12°58′N	894	10	16	PPC
KE	Kerela, India	76°38′E	10°30′N	127	4	14	PPC
XL	Siem Reap, Cambodia	103°50′E	13°22′N	16	5	15	PPC
NHG	Siem Reap, Cambodia	103°58′E	13°35′N	42	5	15	PPC
Phi	Iligan, Philippines	124°10′E	8°10′N	107	9	9	PPC
MaL	Melaka, Malaysia	102°21′E	2°22′N	50	7	8	PPC
SL	Central Province, Sri Lanka	80°25′E	7°11′N	451	10	14	PPC
BA	Bali, Indonesia	115°11′E	8°24′S	490	4	4	PPC
Native region							
BRO	Broward, Florida, USA	80°06′W	26°08′N	5	4	8	PPC
MAR	Martin, Florida, USA	80°15′W	27°06′N	5	4	8	HITBC 135,949
MD	Miami, Florida, USA	80°20′W	25°38′N	5	4	8	PPC
FAK	Collier, Florida, USA	80°29′W	25°52′N	5	4	6	HITBC 135942
CB	Pinar del Rio, Cuba	82°50′W	22°45′N	565	7	7	HITBC 135940
Jam	St Andrew, Jamaica	76°43′W	18°02′N	747	5	5	PPC
PP	Ponce, Puerto Rico	66°51′W	18°11′N	300	10	10	HITBC 135945
PM	Manati, Puerto Rico	66°47′W	18°47′N	50	10	13	PPC
MA	Mamoral, Trinidad	61°17′ W	10°27′ N	63	10	10	HITBC 135947
FE	Felicity, Trinidad	61°25′W	10°31′N	10	10	10	HITBC 135944
Tob	Franklyn, Tobago	60°46′W	11°13′N	18	6	10	PPC
Trin	Santa Cruz, Trinidad	61°30′W	10°43′N	139	6	10	PPC
Coy	Chiapas, Mexico	93°09′W	16°44′N	640	9	10	HITBC 135938
Las	Quintana Roo, Mexico	88°47′W	18°38′N	–	10	10	HITBC 135943

Table 1 continued

ID	Location	Longitude	Latitude	Altitude (m)	N_{DNA}	N_{SSR}	Specimen accessions
Mic	Michoacan, Mexico	103°37′W	18°51′N	950	10	10	HITBC 135948
Teo	Veracrus, Mexico	96°58′W	19°23′N	1,160	10	10	HITBC 135941
Cdv	Tamaulipas, Mexico	99°11′W	23°40′N	600	10	10	HITBC 135939
Ageratina adenophora (Outgroup)							
XS	Yunnan, China	102°37′E	24°58′N	1,932	1	–	HITBC 135935

N_{DNA} and N_{SSR} are sample sizes for sequencing and microsatellite analysis respectively

m metre, *HITBC* Herbarium of Xishuangbanna Tropical Botanical Garden, *PPC* Lab of Plant Phylogenetics and Conservation in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences

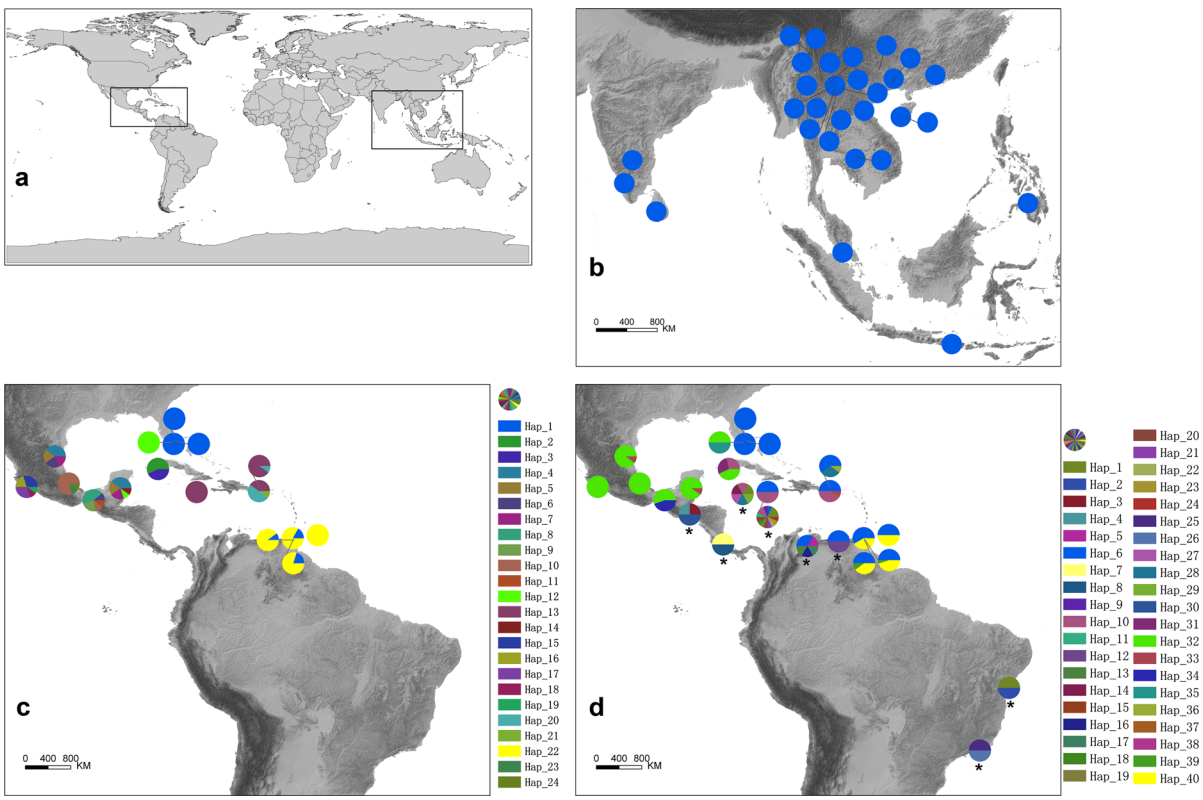

Fig. 1 Sampling sites and haplotype distribution. **a** Sampling sites in native and invasive regions (Asia). **b** Distribution of haplotypes of cpDNA and ITS in Asia. **c** Distribution of cpDNA haplotypes in native regions. **d** Distribution of ITS haplotypes in native regions, an *asterisk* under the *circle* indicates ITS sequences of that population (corresponding to those in supplementary material 1) were obtained from Genbank

(indicated by double peaks) where more than one peak was apparent in the chromatograms of ITS sequences.

Since ITS sequences showed heterozygous sites solely in native populations through direct sequencing (see Results), we further compared the intra-individual diversity of ITS sequences between native and introduced populations by cloning three individuals (indicated by double peaks at some sites) from native population (population PP in Table 1) and three individuals from introduced population (population ML). The cloning protocol followed Yu and Li (2011), and ten positive clones for each individual were sequenced. In addition, 20 ITS sequences from populations in native regions (Central and South America) and the two ITS1

Table 2 Primers of selected molecular markers used in sequencing and microsatellite analysis

Locus name	Primer sequence (5′–3′)	Ta (°C)	Repeats	Source
*atp*B-*rbc*L	*atp*B: ACATCKARTACKGGACCAATAA	50	–	(Chiang et al. 1998)
	*rbc*L: AACACCAGCTTTRAATCCAA			
*psb*A-*trn*H	*psb*A:GTTATGCATGAACGTAATGCTC	50	–	(Sang et al. 1997; Tate 2002)
	*trn*H2:CGCGCATGGTGGATTCACAATCC			
ITS	ITS4: TCCTCCGCTTATTGATATGC	56	–	(White et al. 1990)
	ITS5 m:GGAAGGAGAAGTCGTAACAAGG			(Sang et al. 1995)
CO26	F: CAGACTGGATCATAAGAA	58	$(TG)_8$ … $(TG)_3$	(Yu and Li 2011)
	R: TTACGTGTAATAGAGCCT			
CO50	F: TACCCTGTTATTCCCACT	60	$(TG)_{10}$	(Yu and Li 2011)
	R: CCTAAGCCTTCTTATTTGAT			
CO65	F: CAGTTATCTTCAACACCCAA	58	$(CT)_7$ … $(CT)_4$ … $(TC)_3$	(Yu and Li 2011)
	R: TTTCCGACTAAACCCATC			
CO115	F: TCGTGGTAGAGCAGAAGA	54	$(AG)_6$ GTT$(AG)_4$	(Yu and Li 2011)
	R: AACTGCCAGATCAGGTTG			
CO189	F: AGAGTAAGCACGAGACCG	60	$(TTTTG)_3$ … $(AG)_9$	(Yu and Li 2011)
	R: AGAACTTTACCTCCCACA			
CO227	F: GTTCGTCACCCTTTTCTC	62	$(GA)_5$ … $(AG)_9$	(Yu and Li 2011)
	R: ATCTGCACTTCATCTTCTTC			

F forward primer, *R* reverse primer, *Ta* annealing temperature

genotypes (A and B) from northern Australia (Scott et al. 1998) were downloaded from Genbank (Suppl. 1). Sequences were aligned using CLUSTAL X (Thompson et al. 1997) before manual editing. All DNA sequences of *C. odorata* were deposited in GenBank with accession numbers JX892121–JX892864.

Microsatellite (SSR) genotyping

Among the 11 polymorphic microsatellite (SSR) markers reported previously (Yu and Li 2011), six most polymorphic microsatellite (SSR) markers (Table 2) were selected for genotyping the 522 individuals of *C. odorata* (155 from the native range and 367 from invaded regions). PCR amplification and product detection procedures followed Yu and Li (2011). Samples that did not amplify in the first run or had obscure peaks were repeated three times before being coded as missing data to ensure all loci had less than 5 % missing data.

Analysis of genetic diversity and haplotype network

The total haplotype diversity (H_D) and nucleotide diversity (π) of cpDNA and ITS were calculated using DNASP v 5.10 (Librado and Rozas 2009). Gene diversity within populations (H_S) and total gene diversity (H_T) for cpDNA and ITS data sets were calculated using the program PERMUTCPSSR v 2.0 (Pons and Petit 1996). For SSR data, the presence of null alleles, large allele dropout and scoring errors due to stuttering were tested in MICRO-CHECKER v 2.2.3 (Van Oosterhout et al. 2004). GENALEX v 6.4 (Peakall and Smouse 2006) was used to calculate the observed heterozygosity (H_O) and expected heterozygosity (H_E). We also calculated the number of alleles (A), and allelic richness (A_R) using FSTAT v 2.9.3 (Goudet 2001).

The ITS haplotype reconstruction was conducted through the algorithms provided by PHASE (Stephens et al. 2001; Stephens and Donnelly 2003) in DNASP v 5.10 (Librado and Rozas 2009) due to the heterozygous sites. NETWORK v. 4.6.0 (www.fluxus-engineering.com) was used to construct haplotype networks of cpDNA and ITS data sets following a Median-Joining method (Bandelt et al. 1999) *Ageratina adenophora* (Spreng.) R. M. King and H.Rob. was chosen as the outgroup in constructing haplotype networks since *Ageratina* is thought to be basal to *Chromolaena* (Schmidt and Schilling 2000). A geographic

distribution of the haplotypes was generated according to the longitude and latitude of the collection sites.

Population genetic structure

All 522 individuals were analysed as multilocus genotypes (MLGs) (the genotype resulting from combining alleles at all six microsatellite loci). First, the genotypic frequency was estimated using ARLEQUIN v 3.0 (Excoffier et al. 2005), and then the information for the MLGs for each population was summarized to generate a geographic distribution map. Since the genetic differentiation among populations in Asia was extremely low (see Results), we pooled all samples from invaded regions into a large group for further population genetic analysis. We categorized populations into invasive and native groups. An analysis of molecular variance (AMOVA) was implemented to partition genetic variation among populations for both DNA sequences and SSR loci in ARLEQUIN v 3.0 (Excoffier et al. 2005) with significance tested using 10^4 permutations as described in Excoffier et al. (1992), the genetic structure of *C. odorata* was assessed by Wright's fixation index (Wright 1949). We also calculated the global F_{ST} (Weir and Cockerham 1984) for populations in invaded and native ranges, respectively. Departures from the Hardy–Weinberg equilibrium (HWE) were analysed using exact tests in GENEPOP v 3.4 (Raymond and Rousset 1995).

Population clustering was tested using a Bayesian clustering method implemented in STRUCTURE v 2.2.3 (Pritchard et al. 2000). The number of expected clusters (K) between 1 and 10 was tested. Fifteen independent runs were performed for each specified K-value to verify convergence. For each run, the admixture model (the default value) was assigned by assuming independent allele frequencies with additional 10^6 generation repetitions of the Markov Chain (MC) after a burn-in of 10^6 generations (Pritchard et al. 2000). The statistic ΔK, based on the rate of likelihood of change between successive K values, was used to estimate the optimal value for K (Evanno et al. 2005).

Results

Genetic structure revealed from cpDNA and ITS

The lengths of aligned sequences of combined cpDNA (*atp*B-*rbc*L and *psb*A-*trn*H) and ITS fragments in *C. odorata* were 1,284 and 683 bp respectively. The ITS sequences of 74 individuals (57 %) in native regions showed heterozygous sites, which was not found in any of 118 individuals from populations in Asia. Seven ITS genotypes were revealed by cloning in three individuals from native population (PP), while only one genotype was found in three individuals from introduced population (ML). Including gaps, there were 77 and 37 polymorphic sites in cpDNA and ITS sequence data sets, respectively, resulting in 24 cpDNA and 40 ITS haplotypes (Figs. 1, 2). The total haplotype diversity (H_D) was 0.685 for cpDNA and 0.596 for ITS, while nucleotide diversity (π) was 0.001 for cpDNA and 0.008 for ITS.

The phylogeographic network analysis of 24 cpDNA and 40 ITS haplotypes (Figs. 1, 2) showed that populations in Mexico contained the most haplotypes of cpDNA, and Jamaica with the most of ITS fragments. All 118 individuals from 29 locations in ten countries across South and Southeast Asia existed as a single haplotype for both cpDNA (h1) and ITS (h6). However, the frequency of this single cpDNA/ITS haplotype in native range of the species was not higher when compared to other haplotypes. The single cpDNA haplotype in Asia was also found in populations from Florida, Trinidad and Tobago in the West Indies, but not in populations from Jamaica which was previously assumed as the possible source location of the introduction. The single ITS haplotype in Asia was also found in Jamaica, Puerto Rico, Venezuela besides Florida, Trinidad and Tobago, but no ITS haplotype sharing was found between Brazil and Asia. The single haplotype of ITS in Asia was further confirmed to be the most common haplotype A found in Australia.

Since all samples from Asia shared the same haplotype of cpDNA and ITS, genetic differentiation (F_{ST}) of cpDNA and ITS among *C. odorata* populations in Asia was virtually zero, while genetic differentiation calculated in ARLEQUIN among populations in the native range was high and significant (cpDNA $F_{ST} = 0.671$, $P < 0.01$; ITS $F_{ST} = 0.693$, $P < 0.01$). Genetic differentiation between the invasive group and the native group was high and significant (cpDNA $F_{CT} = 0.376$, $P < 0.01$; ITS $F_{CT} = 0.447$, $P < 0.01$) (Table 3). The AMOVA results revealed that the majority of variation was found between invasive and native group and among populations within native group (cpDNA: 37.55 % and 37.74 %; ITS: 44.73 % and 32.86 %) (Table 3).

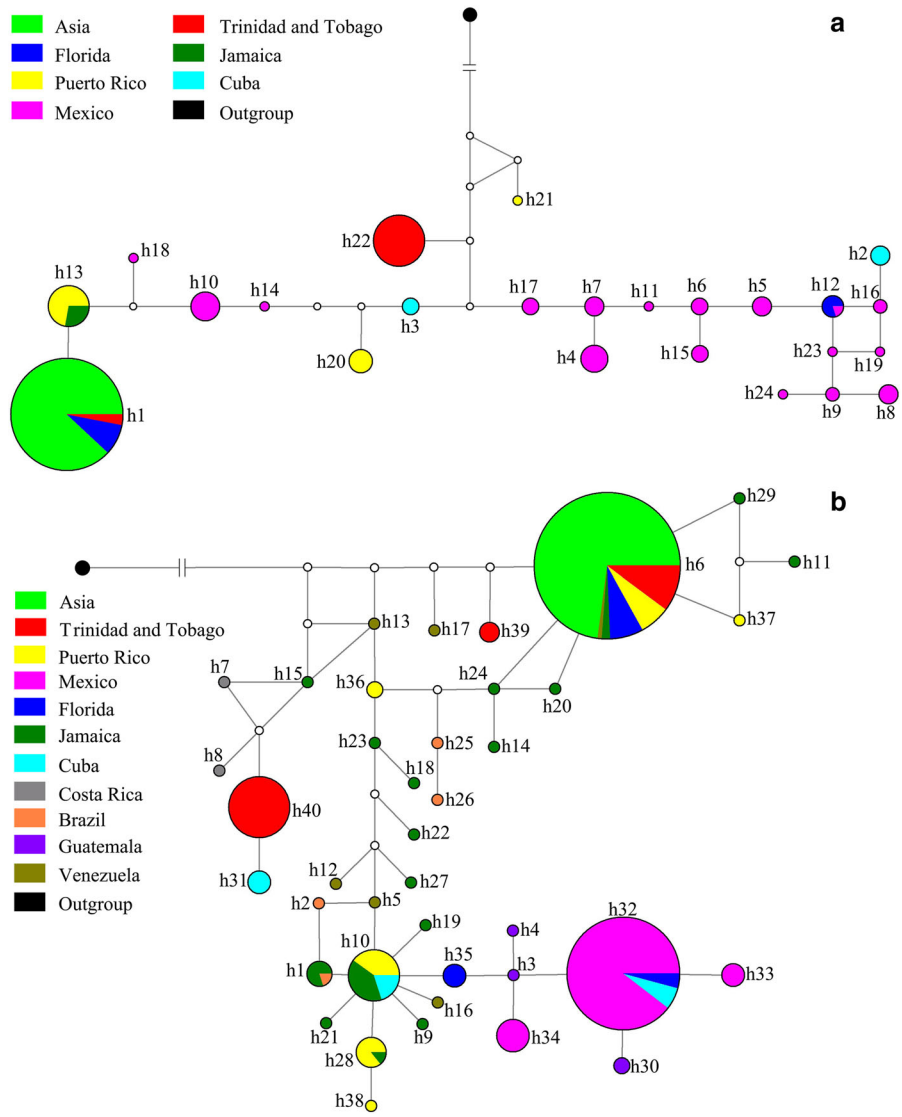

Fig. 2 The cpDNA (**a**) and ITS (**b**) haplotype network. Sampled haplotypes are indicated by *circles* and missing or unsampled haplotypes are indicated by *white circles*. Haplotypes are *colored* according to sites from which the samples were collected. *Circle* size is proportional to the observed haplotype frequency. *Ageratina adenophora* is the outgroup

Gene diversity within populations (H_S) and total gene diversity (H_T) in the native populations was 0.350 and 0.917 for cpDNA and 0.556 and 0.866 for ITS. In contrast, H_S and H_T were zero in populations in Asia for both cpDNA and ITS.

Genetic structure revealed from microsatellite DNA (SSR)

Analysis with MICRO-CHECKER suggested no large allele dropout or scoring errors in our data set, while null alleles were likely to be present at five out of the six loci in several (ranged from one to four) populations in the native regions with the null allele frequency >0.1. Significant deviations from HWE were observed at multiple loci and at a few populations ($P < 0.01$) (Suppl. 2). The observed heterozygosity (H_O) in the Asian group was either zero or one, while H_O ranges from 0.104 to 0.649 in native populations (Table 4). The mean observed heterozygosity (*Ho*) of Asian populations (0.335 ± 0.210) was slightly lower than that in native populations (0.422 ± 0.088). However, the mean expected heterozygosity (H_E) for Asian populations (0.169 ± 0.105) was significantly lower than that in native populations (0.753 ± 0.041). Both the number of alleles (A) and allelic richness (A_R) of six SSR loci in the Asian group ranged from 1 to 4 (average = 2.3 ± 0.5), while A and A_R varied from 6

Table 3 Results of the analysis of molecular variance (AMOVA) for cpDNA, ITS and SSR loci of *Chromolaena odorata*

Locus	Source of variation	d.f.	SS	VC	Variation (%)	Fixation indices
cpDNA	Among groups	1	302.160	2.30	37.55	$F_{CT} = 0.376$*
	Among populations within groups	44	604.112	2.31	37.74	$F_{SC} = 0.604$*
	Within populations	201	304.400	1.51	24.70	$F_{ST} = 0.753$*
	Total	246	1,210.672	6.13		
ITS	Among groups	1	438.397	1.59	44.73	$F_{CT} = 0.447$*
	Among populations within groups	52	635.634	1.17	32.86	$F_{SC} = 0.595$*
	Within populations	480	384.399	0.80	22.40	$F_{ST} = 0.776$*
	Total	533	1,458.431	3.57		
SSR loci	Among groups	1	266.982	0.59	35.08	$F_{CT} = 0.351$*
	Among populations within groups	44	343.561	0.31	18.36	$F_{SC} = 0.283$*
	Within populations	998	787.122	0.78	46.56	$F_{ST} = 0.534$*
	Total	1,043	1,397.665	1.69		

Populations were categorised into two groups (invasive and native group)

df degree of freedom, *SS* sum of squares, *VC* variance components, F_{CT} genetic differentiation among groups, F_{SC} genetic differentiation among populations within groups, F_{ST}, genetic differentiation index

Levels of significance: * $P < 0.01$

to 16 and 5.9 to 16.0, respectively, with an average of 10.8 ± 1.7 in native populations (Table 4). Genetic differentiation (F_{ST}) calculated among *C. odorata* populations in Asia was not significantly deviated from zero ($F_{ST} = -0.033$, $P > 0.01$), while genetic differentiation among native populations was high and significant ($F_{ST} = 0.522$, $P < 0.01$). AMOVA based on six microsatellite loci indicated that 46.56 % of the variation partitioned within populations (mainly from native regions), 35.08 % to between invasive and native group, 18.36 % to among populations within each group. The genetic divergence between the invasive group and the native group was significant ($F_{CT} = 0.351$, $P < 0.01$) (Table 3).

In total, 71 MLGs were identified across 522 samples, among which five were from Asia and 67 from native regions. Notably, a single predominant MLG comprised 98.9 % of the samples in Asia, with only four individuals showing different MLGs. The predominant MLG in Asia was shared by three populations from Florida and all four populations from Trinidad and Tobago. However, this MLG was not present with significantly higher frequency in the native range comparing to other MLGs (Fig. 3). There was no MLG sharing between Jamaica and Asia. Mexican populations contained most of the MLGs (50 out of 71), which is consistent with the results of cpDNA haplotype network analysis. Bayesian clustering analysis revealed that the optimal K was 2 (invasive and native clusters) based on the ΔK trend, while the next most likely K was 7 (only more clusters within native populations) (Suppl. 3). The genotype of Asian *C. odorata* was virtually identical except in four samples. The dominant genotype in introduced populations was also found in the native populations from Florida, Trinidad and Tobago, which is congruent with results from cpDNA and ITS sequence data sets (Fig. 4).

Discussion

The presence of a single haplotype of cpDNA and ITS fragments, combined with one predominant multilocus genotype based on six microsatellite loci in samples spreading over a vast geographic area, indicates that *C. odorata* might exist as a single lineage in Asia. Taking together with the findings from physiological experiments (Qin et al. 2013), our results suggested that the dominant genotype found in Asia was likely to be an invasive genotype that might be more invasive than others. This genotype is likely to be the result of post-invasion selection of genotypes with higher competitive ability in invaded

Table 4 Genetic diversity for invasive and native group of *Chromolaena odorata* based on six SSR loci

Locus	Asia					Native				
	N	H_O	H_E	*A*	A_R	*N*	H_O	H_E	*A*	A_R
AC26	356	0.000	0.000	1	1.0	152	0.211	0.701	6	5.9
AC50	360	1.000	0.500	2	2.0	154	0.565	0.819	10	9.9
AG65	360	0.003	0.006	3	3.0	143	0.462	0.788	11	11.0
AG115	363	0.000	0.000	1	1.0	154	0.104	0.578	7	6.9
AG189	367	0.005	0.008	3	3.0	154	0.649	0.865	15	14.9
AG227	366	1.000	0.503	4	4.0	152	0.539	0.766	16	16.0
Mean	362	0.335	0.169	2.3	2.3	152	0.422	0.753	10.8	10.8
SE	1.7	0.210	0.105	0.5	0.5	1.7	0.088	0.041	1.7	1.7

N number of samples, H_O observed heterozygosity, H_E expected heterozygosity, *A* number of alleles, A_R allelic richness, *SE* standard error

regions. Our results also suggested that it is likely that only one single introduction of *C. odorata* occurred in Asia, and that the most likely geographic origin of this introduction was Trinidad, Tobago and adjacent areas in the West Indies.

Successful invasion of *C. odorata* in Asia

Colonizing a novel environment represents a genetic challenge to invasive species because the species have to confront new selective pressures in the new environment (Pérez et al. 2006). A new selection regime acting on the invaders may involve intensified selection for adaptive genotypes and/or relaxed selection for defense because of the absence of coevolved natural enemies (Hänfling and Kollmann 2002). It was previously thought that reduced genetic diversity would limit the ability of introduced populations to evolve in new habitats (Allendorf and Lundquist 2003). However, some studies have demonstrated that the successful invaders may not necessarily have high genetic diversity (e.g. Tsutsui 2000; Poulin et al. 2005; Zepeda-Paulo et al. 2010; Zhang et al. 2010).

Using ISSR markers, Ye et al. (2004) found very low genetic variation in *C. odorata* from southern China. Our study consistently revealed extremely low genetic diversity of *C. odorata* across vast areas of tropical and subtropical Asia, evidenced by the single haplotype of cpDNA and ITS fragments (Figs. 1, 2) and the single predominant MLG of six SSR loci (Fig. 3). This pattern of genetic diversity might be mainly caused by the founder effect as suggested by Ye et al. (2004). The comparison of sequences revealed that the single haplotype of ITS in Asia was also the most common haplotype A (more widespread form) found in Australia. Our sampling sites in Asia spanned from latitude 8°S–25°N and longitude 76°E–124°E, covering most of the tropical part of Asia. This vast geographic distribution indicates that *C. odorata* is adaptive to diverse/heterogeneous habitats and environments. *C. odorata* is also reported to be capable of clonal propogation (Gautier 1993; Liu et al. 2006 and our filed observation), which might be one important factor for the successful invasion of this species as proposed by Ye et al. (2004). There are several lines of evidence supporting this scenario: (1) the observed heterozygosity (H_O) in the Asian group was either zero or one (Table 4), all the individuals in invasive populations were heterozygotes in two of the six SSR loci, indicating that sexual propagation might have been rare; (2) the expected heterozygosity of Asian populations was significantly lower than that in native populations. In contrast, the observed heterozygosity (*Ho*) of Asian populations was similar to that of the native populations (Table 4), this pattern again could be a result of clonal propagation of *C. odorata* in Asia but sexual reproduction as the major productive mode in native regions. The population density in native regions is significantly lower than that in Asia (Y-L. Zheng, personal communication). Taken together, our results provide evidence that *C. odorata* exists in Asia as a single well-performing genotype, and likely adaptive to different microhabitats through phenotypic plasticity. For instance, the species was observed as an undershrub-like growth form in open fields while a liana-like growth form at the forest edge (Q-M. Li, personal observations).

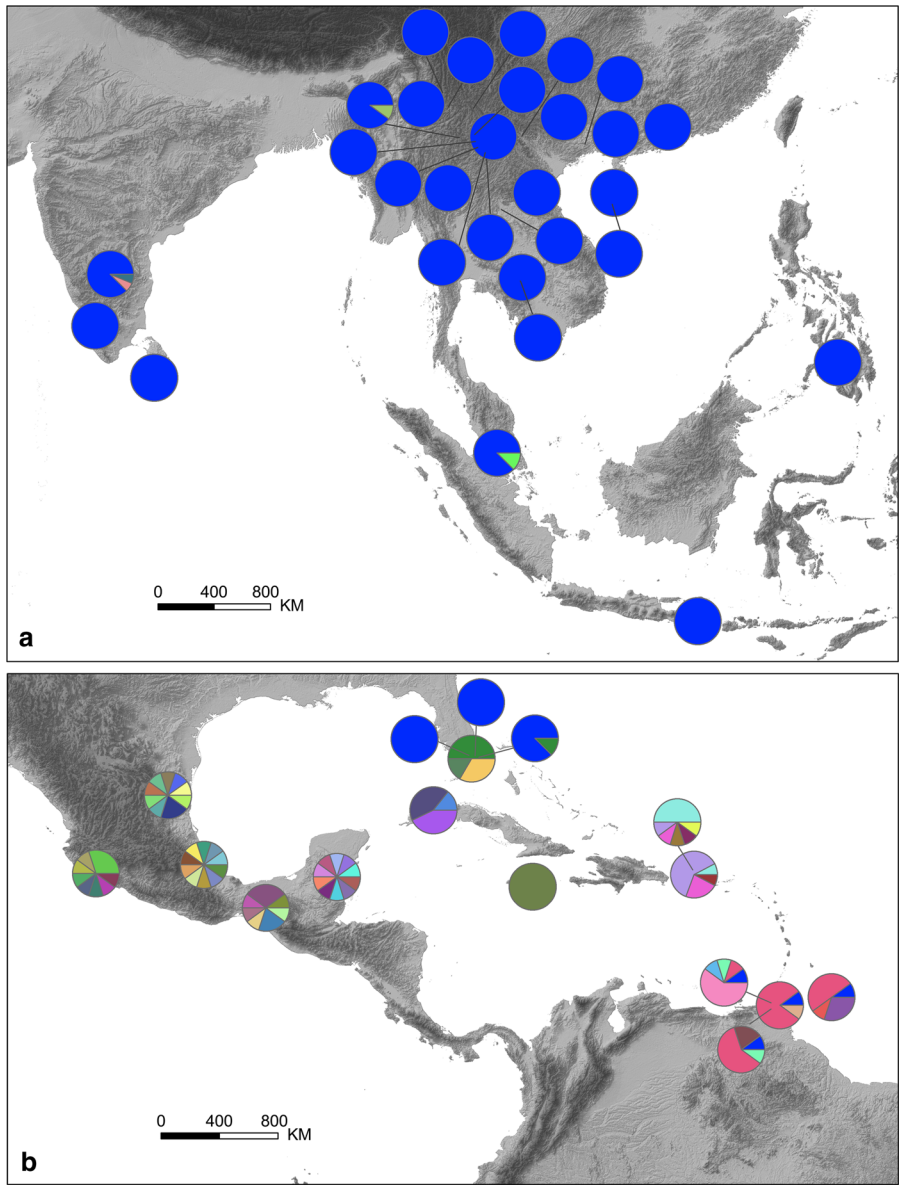

Fig. 3 Frequency of multilocus genotypes (MLGs) of *C. odorata* in the invaded and native regions. Each *circle* represents an analysed population, indicating the relative frequency of each MLG. *Different colours* in the *circular graphs* indicate different MLGs

Successful invasion might be facilitated by better adaptive ability of particular invasive genotypes (Fuentes-Contreras et al. 2004; Le Roux et al. 2007; Zepeda-Paulo et al. 2010; Harrison and Mondor 2011). Genotypes with stronger competitive ability and novel aggressive genotypes generated through recombination have been considered as possible explanations for the existence of well-performing genotypes (Ellstrand and Schierenbeck 2000; Allendorf and Lundquist 2003; Lavergne and Molofsky 2007; Zhang et al. 2010). The dominant invasive genotype of *C. odorata* found across Asia is unlikely to be the result of bias in sampling as our collecting sites cover vast geographic regions of Asia. The explanation of novel genotypes generated through recombination among these genotypes was not supported here either, since the dominant genotype across Asia was also found in Florida, Trinidad and Tobago in native regions (Figs. 1, 2). Therefore, our study suggest the existence of genotypes with stronger competitive ability facilitated successful invasion of *C. odoarta* in Asia.

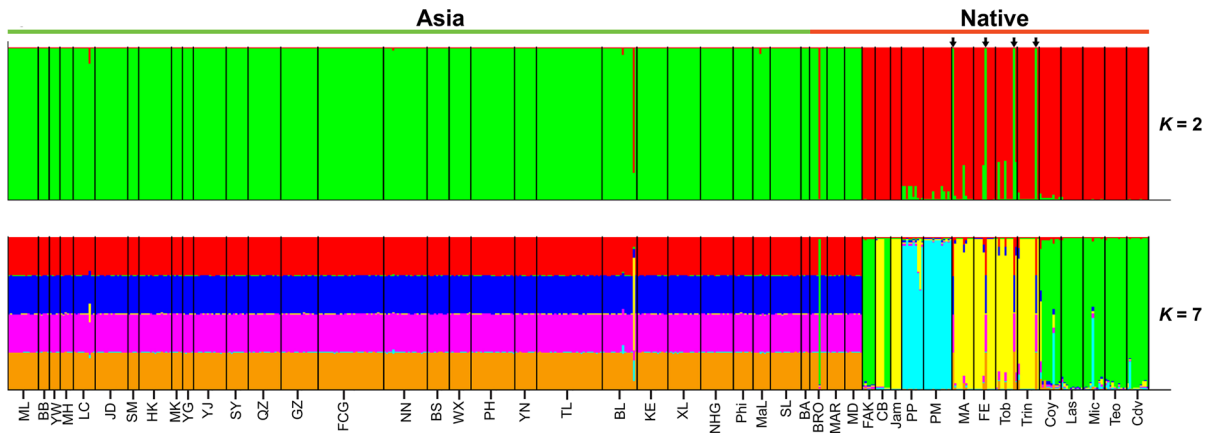

Fig. 4 Bayesian inference analysis of nuclear microsatellite DNA data performed in STRUCTURE. Results of both two and seven clusters were shown; *arrows* marked the invasive genotype detected in Florida, Trinidad and Tobago populations

Although only a single haplotype of cpDNA and ITS was found in *C. odorata* across Asia, multiple genotypes or MLGs were identified based on SSR data set (Figs. 3, 4). Other genotypes with very low frequency besides the predominant one were found in three populations (BL from India, LC from China and MaL from Malaysia) in Asia. It is therefore likely that a considerable number of individuals with different genotypes of *C. odorata* were initially introduced to Asia, but only the genotype with stronger competitive ability has been selected in the new environment. Similar scenario was also proposed in *Eichhornia crassipes* (Mart.) Solms (Ren et al. 2005; Zhang et al. 2010). Note that there was no climatic niche shift of *C. odorata* among Asia, Australia and America (Kriticos et al. 2005), suggesting the concordant climatic tolerance of *C. odorata* between native and invasive regions (Asia and Australia). However, this invasive genotype (i.e. haplotype A of ITS1) of *C. odorata* was suggested to show a wider distribution compared to the other genotype in northern Australia (Scott et al. 1998). Moreover, individuals with the predominant genotype of *C. odorata* has shown stronger competitive ability compared with those from native regions under high nutrient treatment (Qin et al. 2013). In conclusion, current evidence supports the argument that some particular genotypes of a species might be more invasive (adapted) than others (Allendorf and Lundquist 2003; Zhang et al. 2010), which could be one important mechanism facilitating the successful invasion of *C. odorata* in Asia, although identifying candidate genes involved with introduction success of this exotic weed demands further investigation.

Geographic origin of *C. odorata* in Asia

Identifying geographic sources of invasion enables direct ancestor-descendent comparisons of phenotypic traits between native and introduced populations (Keller and Taylor 2008; Colautti et al. 2009), that were valuable for investigating the ecological factors underlying successful invasion. Jamaica was presumed as the source of the first *C. odorata* plants introduced into the Calcutta Botanic Garden in India (McFadyen 1993). However, this speculation is supported neither by a recent study (Paterson and Zachariades 2013) nor by our analysis using multiple DNA markers, as the homogeneous haplotype of cpDNA and ITS and the predominant SSR MLG were not found in populations from Jamaica. Our results, based on the sharing of both the haplotype and MLG between native and invasive populations, suggested that the single dominant invasive genotype in Asia was likely to have originated from Florida, Trinidad or Tobago. Almost identical findings were achieved by Paterson and Zachariades (2013) recently. Numerous historical records suggested that *C. odorata* in Asia was likely to be introduced from the West Indies (Biswas 1934; Bennette and Rao 1968; McFadyen 1993). We therefore propose that the source location of Asian *C. odorata* was more likely Trinidad, Tobago and adjacent areas, than Florida.

The invasion genetics of *C. odorata* in Asia has also shed light on the introduction history of this species. Historical records suggested two independent introductions of *C. odorata* to Asia (to India and to Singapore/Malaysia) from the West Indies, separated by some 75 years (Biswas 1934; Bennette and Rao 1968; McFadyen 1989; Muniappan et al. 2005). Multiple introductions have been proven to increase total genetic diversity of several invasive species (Genton et al. 2005; Marrs et al. 2008; Pairon et al. 2010). The extremely low genetic diversity in *C. odorata* across the vast invaded areas of Asia implies that a single introduction from the West Indies to Asia is more likely than separate independent introductions, supporting the argument by some researchers (Gautier 1992; McFadyen 1993; Zachariades et al. 2009). The single haplotype of ITS in Asia was also the most common haplotype A found in Australia, implying that the more widespread form of *C. odorata* in Australia was likely from Asia other than from its native regions. We speculate it was from Indonesia (East Timor), aided by the movement of personnel and equipment during the Second World War, as previously suggested by some researchers (McFadyen 1989; Muniappan and Bamba 2000; McFadyen 2002).

Management implication and future study

Biological control has been recognized as an efficient way to reduce the current and potential impact of *C. odorata* in tropical areas (Goodall and Erasmus 1996). For example, the stem-galling tephritid fly *Cecidochares connexa* (Macquart), one of the natural enemies of *C. odorata*, has been introduced from South America and used as a potential bio-control agent in Indonesia, the Philippines and Guam (Muniappan et al. 2002; McFadyen et al. 2003; Cruz et al. 2006), though the efficacy remains to be seen. Given that introduced populations of *C. odorata* in Asia are comprised by a single haplotype and a predominant MLG, importing locally adapted natural enemies from Trinidad and Tobago (the most likely source locations) may be considered effective in controlling this weed in Asia (Müller-Schärer et al. 2004). However, extra caution is needed since biological control agents may also become self-defeating (Garcia-Rossi et al. 2003; Zalucki et al. 2007).

The contrasting pattern (limited distribution in the native range and wide distribution in invaded regions of the invasive genotype) indicates differentiated adaptive capacity of *C. odorata* in different environment. Some important physiological factors that promote the invasion of *C. odorata* in Asia have been reported recently (Qin et al. 2013). Future work should investigate the underlying mechanisms that give rise to the invasive genotype with a stronger competitive ability. This could possibly be achieved through common garden experiments for direct ancestor-descendent comparisons of physiological traits between native and introduced populations and determining the molecular genetic basis by genomic approaches, such as identifying genes that are involved in introduction success (Prentis et al. 2010; Hodgins et al. 2013).

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