



Original article

Genetic variation and spread pattern of invasive *Conyza sumatrensis* around China's Three Gorges Dam[☆]Ming-Xun Ren^a, Xiao-Qiong Li^{a,b}, Jian-Qing Ding^{a,*}^aLaboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, the Chinese Academy of Sciences, Moshan, Wuchang District, Wuhan 430074, China^bGraduate school of the Chinese Academy of Sciences, Beijing 10093, China

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ABSTRACT

Genetic diversity and structure within and between 17 populations of invasive *Conyza sumatrensis* (Asteraceae) around the world's biggest hydroelectric dam (Three Gorges Dam (TGD) on the Yangtze River in China) and nearby localities were surveyed using inter-simple sequence repeat (ISSR) markers to determine the spread pattern of this invader in TGD and nearby regions. A total of 434 individuals were analysed, for which 15 ISSR primers amplified 81 bands, with 54 (66.7%) being polymorphic. The percentage of polymorphic loci within a population ranged from 31% to 58%, Nei's gene diversity was 0.385 ± 0.056 , and mean Shannon's Index was 0.5815 ± 0.0833 , indicating a high genetic variation in this self-fertile plant. Mass seed production and multiple introductions associated with dam construction and local development were thought to be responsible for the high level of genetic variation. Analysis of Molecular Variance revealed 36.5% of genetic variation residing within populations, 35.0% among populations within regions, and 28.5% among the three regions: TGD, upper reaches of TGD, and lower reaches of TGD. Most populations were genetically related to their nearest neighbors, while gene flow (mainly via seed movement) across TGD existed. Long-distance dispersal of seeds and pollen such as by water current, wind and human transportation could explain the low level of geographic structure of genetic variation. The highest genetic variation was found in a population in TGD, and most populations from TGD showed closer genetic relationship to the lower reaches population, which indicated that *C. sumatrensis* at TGD has likely experienced multiple introductions mainly from lower reaches, which is near the area of primary introduction (southern China) of *C. sumatrensis*.

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1. Introduction

One core question in invasion ecology is to figure out the source (s) of invasive populations and their spread patterns (Buchan and Padilla, 1999; Mack et al., 2000; Jongejans et al., 2008). Therefore, genetic studies of invasive plants are becoming increasingly common (e.g. Carter and Sytsma, 2001; Ren et al., 2005; Ward et al., 2008), because reliable information of introduction histories is usually difficult to obtain without analyses of population genetic structure. Human-mediated dispersal such as vehicle transportation and intentional long-distance introduction is acknowledged as the main factor facilitating the expansion of many invasive plants, particularly their rapid and disjunct spread over long distances (Buchan and Padilla, 1999; Mack et al., 2000; Sakai et al., 2001; Ren et al., 2005). But natural forces such as

water current and wind dispersal of propagules can also play an important role in the expansion of invasive populations (Cohen and Carlton, 1998; Pan et al., 2006; Ren and Zhang, 2007; Jongejans et al., 2008). Therefore, the genetic variation and structure of invasive populations reflect an outcome of interactions of these factors (Lee, 2002; Lambrinos, 2004; Ren et al., 2005). Usually, genetic similarity of long-distanced populations is explained mainly by human-mediated dispersal, while close genetic relatedness to nearest neighbor is largely attributed to the natural spread of propagules (Carter and Sytsma, 2001; Ren et al., 2005; Dlugosch and Parker, 2008a,b).

Three Gorges Dam (TGD) on the Yangtze River in China is the world's largest hydroelectric dam (Wu et al., 2003), forming the world's biggest reservoir area (Three Gorges Reservoir Area, TGRA) with a length of >660 km and an area of about 78,000 km² (Wu et al., 2003). TGRA fragments large, continuous habitats into smaller patches through flooding and dam-related activities such as construction of new cities and roads, which can also import alien plants into TGRA and/or accelerate the expansion of invasive populations in this area (Wu et al., 2003; Ding et al., 2008).

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After completion of the TGD in 2006, many invasive plants were found near the dam wall, especially the South America native (Li and Xie, 2002), alien fleabane *Conyza sumatrensis* (Asteraceae) (Ding et al., 2008). *C. sumatrensis* is an annual or biennial tall herbaceous plant (Thebaud et al., 1996; Case and Crawley, 2000). This species has one erect main stem with several branches with an apical panicle of capitula on the top (Thebaud et al., 1996). It reproduces solely by mainly self-pollinated seeds (Case and Crawley, 2000; Hao et al., 2009), with no clonal growth known in this weed. Due to mass seed production, seed pappus facilitating wind and water dispersal and generalized pollination mode (Case and Crawley, 2000), this species becomes invasive in many subtropical and temperate zones (Thebaud et al., 1996; Case and Crawley, 2000; Ding et al., 2008). In China, this species is mainly distributed in southern and central parts of the country, apparently in disturbed areas around cities. Before the construction of the TGD, this invasive species was only reported in several big cities along the Yangtze River such as Chongqing, Yichang, and Wuhan (Li and Xie, 2002; M.X. Ren, personal observation). Recently, this species has been claimed as the most common terrestrial weed around TGD (Ding et al., 2008). It mainly distributed near towns on the Yangtze River and no more than 1 km away from the river because there are steep high lands with rocks, not suitable for this tropical weed. The vast coverage by this noxious species near TGD and nearby towns has caused serious environmental problems and economic losses in this area (Ding et al., 2008; Weber et al., 2008), and even threatens species diversity and ecosystem health in this species-rich region which was considered as “hot-spot of biodiversity” (Li and Xie, 2002; Wu et al., 2003) and one of the “distribution centers of China’s endemic species” (Ying and Zhang, 1984).

TGD has experienced extensive traffic due to dam construction and local economics, so human-mediated dispersal of *C. sumatrensis* in the site is possible. In addition, the Yangtze River is the third biggest river in the world (Wu et al., 2003) and water current is large and many branch rivers join it at the Three-Gorges section. Consequently the dispersal of invasive species through water current, wind or other natural forces is also very likely. Nevertheless, the relative contributions of the human-mediated dispersal vs. natural spread for the invasion of *C. sumatrensis* are poorly documented.

Here we report our research on patterns of genetic diversity of 17 *C. sumatrensis* populations, 5 at TGD and the others from nearby main cities, by Inter-simple Sequence Repeats (ISSR) to explore the genetic variability and relationships of these populations. Our particular interest was to investigate where the invasive populations at TGD came from and to estimate the spread patterns of this invasive species and the relative contributions of natural spread (e.g. water current) vs. human-mediated long-distance dispersal (e.g. vehicle transportation due to dam construction or local development). We hypothesized that the *C. sumatrensis* populations at TGD have higher genetic variation than the populations at both lower and upper reaches of TGD, and that this resulted mainly from multiple introduction due to extensive traffics associated with human traffic and construction-associated disturbance.

2. Methods

2.1. Sampling methods

In August 2007 and 2008, we sampled 5 populations of *C. sumatrensis* around the TGD (D1–D5, see Fig. 1), at least 2 km apart. The populations were discrete patches, isolated either by roads and rivers or by residential areas. Each of these populations covered an area of >100 m² and consisted of more than 1500 individuals. Then, we sampled plants at 6 sites (major cities where large *C. sumatrensis* populations were found) each at the upper and lower reaches of

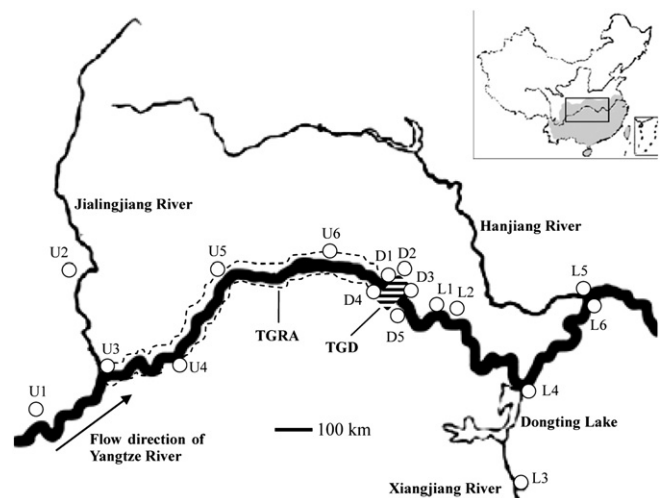


Fig. 1. Geographic locations of the Three Gorges Dam (TGD), Three Gorges Reservoir Area (TGRA), and nearby main rivers. The sampling sites, mainly at suburb of city or big county, are indicated with open circles. Shaded area in the map at top right corner is the current distribution of *Conyza sumatrensis* in China.

TGD, with a geographic distance of >50 km between each others (Fig. 1). In each sampling site, one large population was selected as sampling population.

For each population, at least 20 individuals covering the whole distributing area were randomly sampled at a spacing of >1 m. About 5 fresh young leaves on the stem top per individual were harvested and placed into a zip-lock plastic bag with c. 20 g silica gel. The samples were taken back to laboratory and stored at room temperature for about 1 month to ensure that they were entirely dried before DNA extraction.

2.2. DNA extraction and ISSR-PCR amplification

The DNA of each sample was extracted following the protocol of a DNA extraction kit (Tiangen Company, Beijing, China). DNA quality and quantity were determined visually under ultraviolet light by comparing with DNA ladder D 2000 (Tiangen Company) on 0.8% agarose gels.

Sixty ISSR primers from Sangon Company (Shanghai, China) were screened for polymorphism on six randomly selected samples from three populations (D1, U1, and L6). Fifteen primers that produced clear and reproducible fragments were selected for further analysis (Table 1). Smear and weak bands were ignored,

Table 1
Primers for ISSR-PCR analysis.

Primers	Sequences (5'–3')	Annealing temperature (°C)	No. of scored bands	Size range of bands (base pairs)
ISSR-1	(GTCT) 4	56	7	350–1800
ISSR-2	(ACGT) 4	56	6	250–2200
ISSR-3	(CAAC) 4	56	5	850–1600
ISSR-4	(GAA) 6	52	4	180–1500
ISSR-5	(GAC) 4 GAG	61	7	200–1500
ISSR-6	(AGA) 5 AG	52	6	220–2000
ISSR-7	(CTT) 6 C	56	3	950–1800
ISSR-8	(CTA) 6	53	4	380–880
ISSR-9	CTA (GTA) 5 G	56	3	1100–2100
ISSR-10	G (ACT) 5	51	6	550–1350
ISSR-11	A (GCT) 5 G	64	6	800–1950
ISSR-12	A (TGC) 6	66	5	650–1200
ISSR-13	C (GTCT) 4	59	7	500–1800
ISSR-14	(CACT) 4 C	59	7	400–2100
ISSR-15	A (GCAC) 4 G	69	5	750–1500

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