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Do the introductions by botanical gardens facilitate the invasion of *Solidago canadensis* (Asterceae) in China?

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Summary

Many invasive plants have long been suspected of firstly being introduced and cultivated by a local botanical garden or nursery and then escaping into the field after adapting to the novel environment. The role of botanical gardens in the spread of invasive plants has not yet been explored experimentally. In this article, we studied the possible roles of two botanical gardens in the spread of invasive *Solidago canadensis* (Asterceae) in China by analysing genetic relationships of invasive and native (United States) populations with intersimple sequence repeats markers. Our results showed a high genetic variation (mean $He = 0.292$) and a large proportion of genetic variation (85.6%) residing within populations. *Solidago canadensis* was possibly introduced firstly into eastern China. The plants from Lushan Botanical Garden showed distant genetic distance from all of the other populations, suggesting that this botanical garden had little effect on the invasion of *S. canadensis*. Populations from Wuhan Botanical Garden in central China, however, showed close genetic relationships with local populations and populations in west and south-west China, suggesting gene exchange between these populations. Thus, risk assessment is critical for plant introduction and conservation, as introductions of alien plants by botanical gardens may facilitate plant invasions, while plants conserved in botanical gardens may be at risk by surrounding plant invasions.

Keywords: genetic variation, diversity, gene flow, garden, horticulture, invasive species, molecular markers, population genetics.

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Introduction

Biological invasions have major impacts on ecosystems and economics (Simberloff *et al.*, 2013). A substantial body of evidence indicates a positive correlation between human introduction and invasion success (Gravuer *et al.*, 2008; Xu *et al.*, 2012; Donaldson *et al.*, 2014). For instance, many alien plants that have potential economic use, for example ornamental and forage usage, are often introduced and transplanted by humans out of their native ranges (Dehnen-Schmutz *et al.*, 2007). After breeding and cultivation, some of these alien plants adapt to the novel environmental conditions, subsequently escape into the wild, and in some cases become invasive (Marco *et al.*, 2010).

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Botanical gardens are often careful in their introduction and cultivation (Dawson *et al.*, 2008). Still, many alien plants have been introduced by botanical gardens for conservation purposes (Chen *et al.*, 2009), scientific research and public education. The intentional and extensive planting of non-native species in botanical gardens theoretically often increases the likelihood of plant survival, escape and establishment in uncultivated areas (Marco *et al.*, 2010; Donaldson *et al.*, 2014). Some previous studies have recognised the potential roles of botanical gardens in the spread of invasive plants (Dawson *et al.*, 2008; Marco *et al.*, 2010; Hulme, 2011, 2014). For example, Hulme (2011) points out that 19 of 34 of the world's worst invasive species were introduced and cultivated by botanical gardens. However, studies experimentally examining the potential contribution of botanical gardens to the spread of invasive plants are limited.

Solidago canadensis L. (Asteraceae), an insect-pollinated clonal plant originating in North America (Werner *et al.*, 1980; Fenesi *et al.*, 2015), was introduced into China as an ornamental plant at Lushan Botanical Garden (LBG, the first botanical garden of China, located on Mount Lushan in Jiujiang City of central China) in the 1930s (Li & Xie, 2002; Wan *et al.*, 2008). *Solidago canadensis* was also introduced in the 1990s by Wuhan Botanical Garden (WBG, in Wuhan City, about 200 km west of LBG). In 1935, Shanghai and Nanjing City in eastern China also began to extensively introduce and grow this alien plant for the horticultural trade (Dong *et al.*, 2006a,b). Recently, this species has become invasive and rapidly spread to uncultivated lands, roadsides, edges of forest and grasslands in more than 10 provinces in eastern and central-southern China (Dong *et al.*, 2006b; Xu *et al.*, 2014), including the cities of the two botanical gardens. Our field observations also found that fields adjacent to botanical gardens such as LBG and WBG were also infested by *S. canadensis*. Using AFLP markers, Zhao *et al.* (2015) compared the genetic structure of *S. canadensis* populations from China and North America and the results showed that the *S. canadensis* populations of China may have originated from distinct native sources. Therefore, our inquiry centred on whether the invasive populations in China, especially the populations around botanical gardens, are the result of an escape from or was facilitated by the botanical gardens. Fortunately, the introduction history of *S. canadensis* in China and in botanical gardens is relatively clearly documented, which made it possible for us to answer the question and explore the spread pattern of this invasive plant by analysing genetic relationships among these populations.

In this article, intersimple sequence repeats (ISSRs) fingerprinting (Godwin *et al.*, 1997) was used to detect the genetic variations of *S. canadensis* populations throughout its distribution areas in China, including populations in the two botanical gardens, LBG and WBG, and two native populations from the USA. The main aims of this study were as follows: (i) to investigate genetic diversity and spread patterns of the invasive *S. canadensis* populations in China and (ii) to explore the possible role of introduction by botanical gardens in the spread of *S. canadensis* in China.

Materials and methods

Sample collection and genomic DNA extraction

In June and July 2009, we sampled *S. canadensis* in Wuhan Botanical Garden and Lushan Botanical Garden (Table 1). In each botanical garden, the individuals at the recorded original transplanted sites were first sampled and the plants from other sites in the botanical garden were also collected. We then sampled *S. canadensis* in 24 localities throughout the main distribution range in China (Fig. 1, Table 1) and two populations from its native ranges, Northampton and Chester in the USA (Table 1).

From each population, individuals were randomly selected at a distance of at least 1 m to reduce the possibility of sampling clones from the same genet. Fresh leaves without damage from the top of each plant were collected and preserved in a zip-lock plastic bag with 20 g silica gel. In total, 701 individuals representing 30 putative populations were collected (Table 1).

Total genomic DNA was extracted using the Tiangen DNA quick plant system, according to the manufacturer's protocol (Tiangen, Beijing, China), from 20 to 30 mg of dried leaf material. DNA quality and quantity were determined visually by comparison with the DNA marker DL2000 on 1% agarose gels. All extractions were then stored at −20°C before the next step.

ISSR-PCR amplification

DNA amplification was carried out in a C-1000 thermal cycler (BioRad, München, Germany). A subset of the ISSR-PCR primers described by Dong *et al.* (2006a), and 30 other random ISSR primers were screened; ten primers produced reproducible and clear banding and were selected for further analysis (Table 2). ISSR-PCR was performed in a volume of 10 μL containing 5 μL Golden easy PCR system (Golden Taq DNA Polymerase; 2× PCR reaction mix), 1 μmol of individual ISSR primer and

Table 1 Location and sample size of the *Solidago canadensis* populations studied

Population	Latitude	Longitude	Habitat	Sample size
AQ (Anqing City)	30°32′26″N	117°00′50″E	Roadside	30
CD (Chengdu City)	30°38′58″N	104°11′04″E	Roadside	31
CQ (Chongqing City)	29°35′37″N	106°46′47″E	Machine factory	37
CS (Changsha City)	28°08′20″N	112°57′41″E	Roadside	18
CT (Changting City)	25°49′43″N	116°21′11″E	Farmland	32
GY (Guiyang City)	26°26′36″N	106°40′21″E	Road side and near the nursery	30
HA (Huaian City)	33°36′24″N	119°03′00″E	Roadside	25
HF (Hefei City)	31°50′53″N	117°12′08″E	Development district	25
HN (Huainan City)	32°45′33″N	116°51′14″E	Roadside and railway side	29
HZ (Hangzhou City)	30°09′56″N	120°15′26″E	Railway side	30
JH (Jinghua City)	29°07′54″N	119°38′50″E	Roadside	26
JJ (Jiujiang City)	29°41′15″N	116°02′32″E	Roadside	27
KM (Kunming City)	24°52′48″N	102°48′01″E	Nursery	16
LBG (Lushan Botanical Garden)	29°32′55″N	115°58′44″E	Understory of Mountain Lushan	16
NB (Ningbo City)	29°50′04″N	121°33′00″E	Roadside	14
NC (Nanchang City)	28°42′34″N	115°50′39″E	Roadside	23
NJ (Nanjing City)	32°08′39″N	118°51′42″E	Suburb roadside	13
PD (Pudong New Area, Shanghai)	31°13′22″N	121°33′08″E	Roadside	28
QP (Qingpu District, Shanghai)	31°09′02″N	121°07′13″E	Roadside	23
TH (Taihe City)	26°52′03″N	114°45′13″E	Highway side	10
WBG (Wuhan Botanical Garden)	30°32′55″N	114°24′57″E	Lowland at suburb of Wuhan City	26
WH (Wuhan City)	30°31′41″N	114°24′14″E	Roadside	28
WZ (Wenzhou City)	28°00′45″N	120°42′16″E	Near construction site	40
YC (Yichang City)	30°42′24″N	111°19′01″E	Car washing station	21
YT (Yingtan City)	28°12′52″N	117°00′36″E	Roadside	27
YX (Yuxi City)	24°23′17″N	102°34′02″E	Nursery	13
YY (Yueyang City)	29°21′30″N	113°05′28″E	Levee and roadside	12
ZZ (Zhengzhou City)	34°46′52″N	113°33′33″E	Pharmaceutical factory	29
USA1 (Northampton, USA)	40°11′58″N	75°05′41″W	Roadside	11
USA2 (Chester, USA)	39°53′28″N	75°40′19″W	Roadside	11

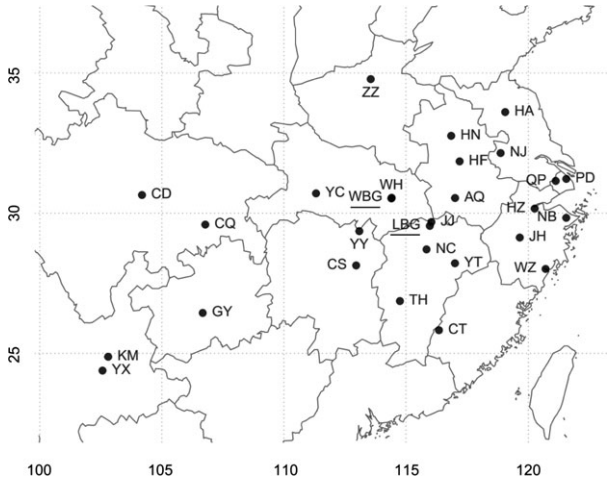

Fig. 1 Geographic locations of 28 *Solidago canadensis* populations sampled in China. The underlined populations are from botanical gardens. The population names are the same as in Table 1.

approximately 5 ng total genomic DNA. The PCR programme involved initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C at 45 s, 50–60°C at 45 s (depending on the primers, Table 2), 2 min at 72°C and a final extension at 72°C for 7 min. Samples were held at 4°C until the next step. By checking contamination, a negative control was included in each amplification. The amplifications were repeated twice, and only clear repetitive bands were used in the data analysis. For ISSR marker profiling, PCR products were subjected to standard horizontal electrophoresis on 1.5% agarose gels in 1× TAE buffer and stained with ethidium bromide. The gels were photographed and recorded using an AlphaImage 2000 auto-imaging apparatus (Alpha Innotech Corp, San Jose, CA, Canada). The sizes of the bands were estimated using DNA ladder DL2000 (Tiangen, Beijing, China).

Statistical analyses

ISSR amplified fragments were designated as present or absent, and a matrix of ISSR phenotypes was assembled. The presence/absence data matrix was analysed with the GenAlEx v. 6.502 (http://biology.anu.edu.au/GenAlEx/) to calculate the percentage of polymorphic ISSR loci (*PPB*), number of alleles (*Na*),

Table 2 The ten intersimple sequence repeat primers used in this study

Primer	Sequence (5′–3′)	Annealing temperature (°C)	Number of scored bands	Size range of bands (base pairs)
UBC-807	$(AG)_8T$	57.2	5	250–2000
UBC-818	$(CA)_8G$	59.6	6	250–1000
UBC-825	$(AC)_8T$	57.2	7	150–1500
UBC-827	$(AC)_8G$	59.6	5	150–2000
UBC-856	$(AC)_8YA$	58.8	4	250–1500
UBC-864	$(ATG)_6$	53.2	5	250–750
ISSR-1	$(CTG)_4CTC$	61.3	4	250–1500
ISSR-9	$(GAA)_6$	53.2	4	250–2000
ISSR-10	$(GAC)_4GAG$	61.3	5	100–1500
ISSR-17	A $(TGC)_6$	66.8	5	300–2000

effective number of alleles (*Ne*), Nei's gene diversity (*He*) and Shannon's diversity index (*I*). To evaluate the genetic structures, we use a Bayesian clustering method with STRUCTURE v.2.3.4 (Evanno *et al.*, 2005). We performed 20 independent runs ($K = 1$–10) with 100 000 Markov chain Monte Carlo (MCMC) after a burn-in period of 25 000 interactions. To best explain the data, ΔK was calculated to identify the optimum number of clusters. The STRUCTURE output was visualised using Structure Harvester. The analysis of molecular variance (AMOVA) and the principal coordinate analysis (PCoA) were carried out using GenAlEx v.6.502 for the correlation of genetic and spatial distances between populations and for the genetic relationships among populations respectively. Nei's genetic distance of plant populations (Nei, 1972) was calculated with GenAlEx v.6.502. The cluster analysis for the populations was performed with the MEGA v.6 (Tamura *et al.*, 2013) based on the Nei's genetic distances using the unweighted pair-group method of averages (UPGMA).

Results

ISSR polymorphism and genetic diversity of S. canadensis

The ten ISSR primers yielded a total of 50 clearly distinct reproducible bands from the 701 individuals of *S. canadensis*. Nei's gene diversity (*He*) was 0.292 and Shannon's diversity index (*I*) was 0.448 at species level (Table 3), suggesting a relatively high genetic variation in this species. Within each of the populations, the percentage of polymorphic bands (*PPB*) with an average of 75.1% varied from 52% for YX population to 92% for JJ population (near the Lushan Botanical Garden). The mean value of *He* and *I* showed similar trends, and minimum and maximum values were both found, respectively, in YX and LBG populations.

Population structure

Based on the AMOVA results, significant genetic difference among populations was detected ($P < 0.001$). Within-populations variation accounted for 85.6% of the total variation in the dataset, while only 14.4% was attributed to among-populations diversity (Table 4). The genetic divergence of populations was not significantly correlated with their geographic distances, as detected by the Mantel test (Mantel test, $r = -0.021$; $P = 0.540$), and the gene flow number was 2.15, suggesting a high level of gene exchange among populations.

The UPGMA analysis based on the Nei's genetic distance showed that the populations were partly mix clustered, although being geographically more distant (Fig. 2). The tree indicates that individuals from WBG were genetically close with the nearby population of WH, and the population of LBG was genetically relatively distant from all the other sampled populations in China. Most of the other populations were not genetically similar to their nearest neighbours (Fig. 2). Two native populations from the USA were genetically similar to PD and AQ populations (both from eastern China) and clustered with the other populations from east China. The populations from central and southwestern China clustered together and then were grouped with the branch of eastern China and native range. Some populations from central China, that is TH, YY, CS and YC, clustered as a branch.

In the STRUCTURE analysis of all populations, ΔK displayed two peaks, $K = 2$ and $K = 5$ (Figure S1). The maximum ΔK was obtained at $K = 2$. At $K = 2$, all populations belonged to the dark cluster, which suggests the populations separated by a relatively small genetic distance and formed one cluster (Fig. 3A, Figure S2). The PCoA, which was performed to determine the consistency of differentiation among populations defined by the cluster analysis, showed the similar results as the clustering analysis (Fig. 3B). The results

Table 3 Genetic variation in 30 populations of *Solidago canadensis* from China and the USA

	Na	*Ne*	*I*	*He*	*PPB* (%)
AQ	1.820 ± 0.055	1.441 ± 0.051	0.394 ± 0.036	0.260 ± 0.026	82.0
CD	1.640 ± 0.074	1.261 ± 0.044	0.265 ± 0.035	0.166 ± 0.024	66.0
CQ	1.740 ± 0.063	1.341 ± 0.052	0.315 ± 0.037	0.204 ± 0.027	74.0
CS	1.640 ± 0.080	1.288 ± 0.040	0.298 ± 0.034	0.188 ± 0.023	68.0
CT	1.620 ± 0.069	1.290 ± 0.047	0.278 ± 0.037	0.179 ± 0.026	62.0
GY	1.680 ± 0.067	1.277 ± 0.047	0.276 ± 0.035	0.174 ± 0.025	68.0
HA	1.800 ± 0.057	1.416 ± 0.051	0.379 ± 0.036	0.248 ± 0.026	80.0
HF	1.800 ± 0.057	1.477 ± 0.052	0.415 ± 0.037	0.278 ± 0.027	80.0
HN	1.860 ± 0.050	1.500 ± 0.054	0.432 ± 0.035	0.287 ± 0.026	86.0
HZ	1.860 ± 0.050	1.459 ± 0.047	0.422 ± 0.033	0.277 ± 0.024	86.0
JH	1.880 ± 0.046	1.493 ± 0.050	0.436 ± 0.034	0.290 ± 0.025	88.0
JJ	1.920 ± 0.039	1.532 ± 0.049	0.466 ± 0.032	0.310 ± 0.024	92.0
KM	1.560 ± 0.071	1.299 ± 0.049	0.278 ± 0.039	0.182 ± 0.027	56.0
LBG	1.780 ± 0.082	1.589 ± 0.053	0.483 ± 0.034	0.330 ± 0.026	86.0
NB	1.760 ± 0.061	1.505 ± 0.052	0.427 ± 0.038	0.290 ± 0.027	76.0
NC	1.800 ± 0.057	1.439 ± 0.052	0.389 ± 0.037	0.258 ± 0.027	80.0
NJ	1.740 ± 0.063	1.371 ± 0.051	0.347 ± 0.036	0.225 ± 0.026	74.0
PD	1.820 ± 0.055	1.443 ± 0.050	0.401 ± 0.035	0.264 ± 0.025	82.0
QP	1.840 ± 0.052	1.532 ± 0.053	0.451 ± 0.035	0.304 ± 0.026	84.0
TH	1.620 ± 0.085	1.439 ± 0.054	0.377 ± 0.040	0.254 ± 0.028	68.0
WBG	1.600 ± 0.070	1.346 ± 0.054	0.302 ± 0.041	0.202 ± 0.029	60.0
WH	1.800 ± 0.057	1.406 ± 0.050	0.377 ± 0.035	0.245 ± 0.025	80.0
WZ	1.680 ± 0.067	1.360 ± 0.054	0.321 ± 0.039	0.211 ± 0.028	68.0
YC	1.720 ± 0.081	1.399 ± 0.052	0.366 ± 0.036	0.239 ± 0.026	78.0
YT	1.840 ± 0.052	1.509 ± 0.050	0.441 ± 0.035	0.296 ± 0.026	84.0
YX	1.500 ± 0.077	1.227 ± 0.041	0.233 ± 0.035	0.147 ± 0.024	52.0
YY	1.620 ± 0.085	1.349 ± 0.047	0.333 ± 0.037	0.216 ± 0.025	68.0
ZZ	1.720 ± 0.064	1.418 ± 0.052	0.368 ± 0.039	0.246 ± 0.028	72.0
USA1	1.760 ± 0.061	1.386 ± 0.046	0.368 ± 0.035	0.239 ± 0.025	76.0
USA2	1.760 ± 0.061	1.375 ± 0.046	0.364 ± 0.034	0.235 ± 0.024	76.0
Average	1.739 ± 0.012	1.406 ± 0.009	0.367 ± 0.007	0.241 ± 0.005	75.1
Species level	2.000	1.483 ± 0.045	0.448 ± 0.029	0.292 ± 0.023	100

Na, observed number of alleles; *Ne*, effective number of alleles; *He*, Nei's (1972) gene diversity; *I*, Shannon's diversity index; *PPB*, percentage of polymorphic bands.
Data are shown as mean ± SE. The population names are the same as in Table 1.

Table 4 AMOVA results of 30 *Solidago canadensis* populations in China as revealed by ten ISSR primers

Source of variation	Df	Sum of squares	Mean sums of squares	Variance components	Percentage of variation	Probability (*P*)
Among Populations	29	898.959	30.999	1.061	14.4%	<0.001
Within Populations	671	4224.733	6.296	6.296	85.6%	<0.001
Total	700	5123.692	37.295	7.358		

of PCoA showed that the first two principal components explained 33.1% and 19.5% of total variation, respectively, and the 69.1% was explained by the first three components.

Discussion

Genetic diversity and structure in S. canadensis

Our ISSR survey of thirty populations of *S. canadensis* revealed a high level of genetic variation in this invasive plant, with a mean *He* of 0.237. The results coincided with previous findings regarding genetic analysis of *S. canadensis* (Dong *et al.*, 2006a; Zhao *et al.*, 2015) and were quantitatively similar to invasive plants such as *Ageratina adenophora* (Sprengel) R. King and H. Robinson (Gui *et al.*, 2009) and *Mikania micrantha* (L.) Kunth. (Wang *et al.*, 2008), but much higher than those of most other clonal invasive plants in China, such as *Eichhornia crassipes* (Mart.) Solms (Ren *et al.*, 2005) and *Chromolaena odorata* (L.) King & H.E.Robins. (Ye *et al.*, 2004).

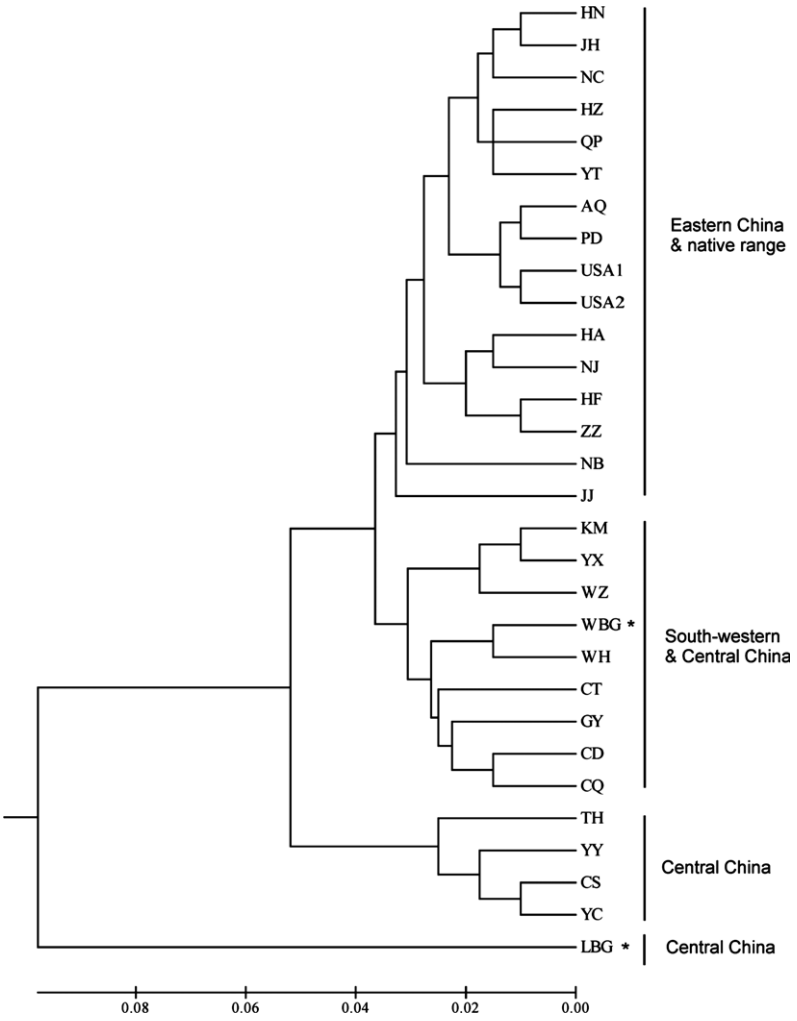

Fig. 2 The UPGMA dendrogram generated by MEGA V. 3.1 for Nei's genetic distances among two *Solidago canadensis* populations from botanical gardens (indicated by *) and 26 invasive populations in China, along with two native populations from the USA. The population names are the same as in Table 1.

The high level of genetic diversity observed may be due to two possible reasons. Firstly, multiple introductions could bring genetic variations into alien populations through the intentional introduction of individuals of different genetic backgrounds (Sakai *et al*., 2001; Dehnen-Schmutz *et al*., 2007; Zhao *et al*., 2015), which could be common in ornamental and horticultural plants (Schierenbeck *et al*., l995; Roux *et al*., 2008). Secondly, additional genetic variations could be accumulated due to sexual recruitment in the field (Ren *et al*., 2005). As an important ornamental and horticultural plant, *S. canadensis* has been widely introduced and bred by arboretums, nurseries and individuals in China (Dong *et al*., 2006b). The observed high level of genetic variation in this plant could be a result of multiple introductions. Furthermore, *S. canadensis* is also a fast-growing and high-yield species due to successful seed reproduction and its genetic variation could be maintained by its sexual reproduction.

The high intrapopulation genetic diversity revealed by AMOVA suggested that sexual reproduction is common in *S. canadensis* populations, which is consistent with previous studies (Dong *et al*., 2006a; Zhao *et al*., 2015). Population genetic structure is an important feature indicating gene flow, mating system in a population and the extent to which populations diverge (Ren *et al*., 2005) and is always affected by a number of factors including historical events, genetic exchange, as well as natural selection (Lee, 2002). In *S. canadensis*, only 14.4% of genetic variation presented among populations and a large proportion of genetic variation (85.6%) resided within populations. Thus, there was little genetic differentiation of *S. canadensis* populations in China, comparable to what has been found in most other clonal plants (Ye *et al*., 2004; Ward *et al*., 2008). Other studies have also showed that invasive clonal plants often maintain low genetic differentiation among populations and relatively high levels of genetic diversity within their populations (Schierenbeck *et al*., 1995; Roux *et al*., 2008; Hagenblad *et al*., 2015). This high level of genetic variation in *S. canadensis* can partially explain the rapid dispersal of this invasive plant, as the richness of

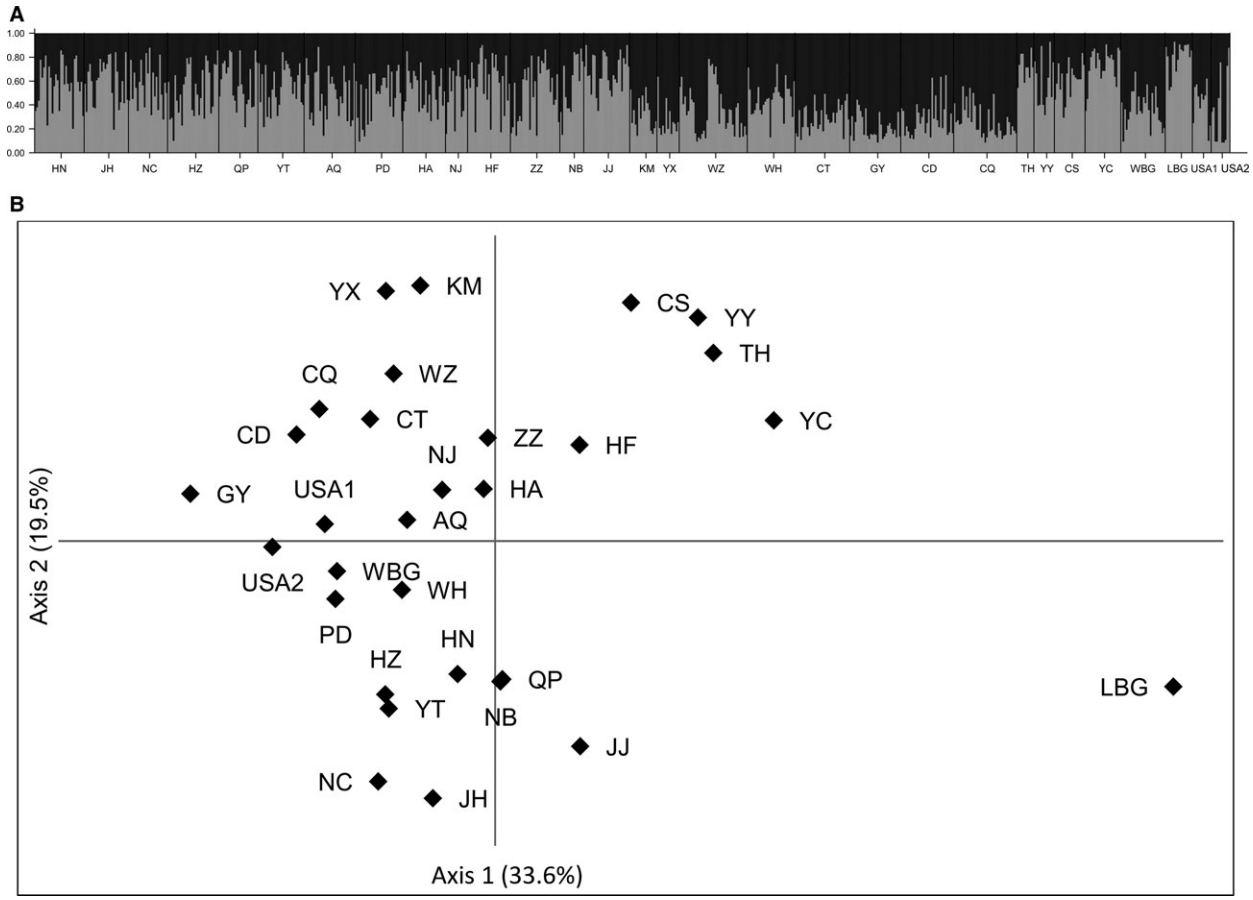

Fig. 3 A) Population structure of *Solidago canadensis* estimated by STRUCTURE ($K = 2$). Each bar represents one individual, and populations are separated by black lines. B) A two-dimensional plot of the principal coordinate analysis (PCoA) derived from ISSR analysis of *Solidago canadensis* populations. The population names are the same as in Table 1.

genetic variation of plant species is normally positively correlated with their adaptive potential (Sakai *et al*., 2001; Prentis *et al*., 2008).

Spreading route of S. canadensis in China

The Mantel test of geographical patterning, as revealed by the UPGMA clusters, indicated that genetic distance between the populations did not broadly reflect the correlation according to their geographic location. Our results indicated that gene flow across large distances had occurred in this invader. Multiple introductions among cities due to anthropogenic factors (such as horticultural trade among the markets, and use by botanical gardens or nurseries) may be the most likely source of such differences (Hulme, 2009, 2011). Still, some nearby populations have clustered together, such as ZZ with HF, HA with NJ, WBG with WH and YX with KM populations (Figs 1 and 2), which we assumed is a result of pollination due to wind and floral visitors.

Population clustering based on Nei's genetic distance between populations and the UPGMA cluster analysis grouped thirty *S. canadensis* populations in China into four main clusters (Fig. 2). The first branch contains most of the populations from east China and two native populations from the USA. The closest genetic relationships between the USA and Pudong, Shanghai (PD) confirmed that *S. canadensis* was possibly first introduced into east China. Also, populations from central China (i.e. WH and WBG) and southwestern China (i.e. CQ, CD and GY) clustered together and then formed a group with clusters containing some south China populations such as KM, YX and WZ populations. Human-mediated dispersal of this invasive plant was probably an important cause of this long-distance dispersal of *S. canadensis*, as it has been widely traded and transplanted as an ornamental plant in central and southern China for nearly two decades (Dong *et al*., 2006b).

Moreover, four geographically close populations (TH, YY, CS and YC) were genetically similar and clustered into a single group, implying that natural dispersal through water or wind over a distant range might take place because the seed pappus enables the small fruits to be buoyant in the wind (Werner *et al*.,

1980), and the pollen flowing via pollinators and the horizontal expansion of clones might also lead to gene flow among nearby populations of *S. canadensis* (Werner *et al.*, 1980). Lastly, populations in LBG showed a distinct genetic relationship with all of the other populations in China and formed a separate cluster. It appeared that there was little gene flow between the population in LBG and China's invading populations, even the surrounding populations such as JJ (Fig. 2), which suggests that although the plant was introduced to LBG very early, the individuals in LBG probably have not escaped from the garden.

The role of botanical gardens in the invasion of S. canadensis

Some research concerning the relation between human introduction and invasion success of plant invaders suggests that there is a high invasion risk of alien plant species when introduced by botanical gardens and arboreta (Dawson *et al.*, 2008; Hulme, 2011). Cultivation of alien plants in botanical gardens often fosters plant adaptation by reducing environmental stochasticity (Mack *et al.*, 2000; Chen *et al.*, 2009) and consequently probably facilitates the escape and spread of these non-indigenous plants. The study of Dawson *et al.* (2008) showed that, in Amani Botanic Garden (Tanzania), among the 214 alien plant species surviving from the original plantings in the early twentieth century, 38 have locally naturalised while 16 have spread widely in the botanical garden, suggesting that this botanical garden has contributed greatly to invasion risks of the alien species. Dehnen-Schmutz *et al.* (2007) report that socioeconomic factors including market presence, plant prices and the date of introduction have important effects on the observed course of invasions. In the process of introduction, plant species chosen for horticultural use are often selected on the basis of early age to maturity, fast-growth, beautiful flowers and high reproductive output, adaptability and a tolerance for various environmental conditions (Dawson *et al.*, 2008). These characteristics that make alien species attractive are the same traits that increase their probability of becoming invasive (Dehnen-Schmutz *et al.*, 2007; Dawson *et al.*, 2008).

Many invasive plants have benefited from cultivation in botanical gardens before escaping into fields, although detailed experimental evidence, such as this study provides, is generally lacking. LBG, which was probably the first place for the introduction in China for *S. canadensis* about 75 years ago (Li & Xie, 2002; Wan *et al.*, 2008), is genetically distant from all of the other *S. canadensis* populations in China and forms a single cluster (Fig. 2). This indicated that the individuals from LBG probably had not escaped from the garden. Therefore, the nearby *S. canadensis* populations (Population JJ) were more likely derived from other sources, such as the populations from eastern China. It is possible that *S. canadensis* has not escaped from the LBG because the LBG is located on the top of Mount Lushan, an isolated mountain covered by dense forest with a long period of fog weather (about 184 days per year) and low average temperature (11.9°C) (Wan & Feng, 2008). This habitat could have limited the growth and reproduction of *S. canadensis* because clonal growth and photosynthesis of this plant are significantly inhibited in such habitats with low light intensities (Sun *et al.*, 2008). Furthermore, it is also possible that the genotype in the LBG population is less aggressive.

Moreover, the results of our experimental study on *S. canadensis* partially supported the above predications. The population from WBG was genetically similar to the local population, which indicated that individuals in WBG might have experienced gene exchanges from the local population. Two scenarios could explain such an observed pattern. First, the transplanted individuals in WBG might have escaped from the botanical garden and established a population in the field or facilitated the establishment and spread of local populations. Second, the plants in WBG may not have escaped but gene flow via seeds or pollen may have taken place between WBG and the surrounding populations; the living plants or rhizomes of *S. canadensis* could even immigrate to the botanical garden by transplanting.

In China, botanical gardens have been set up extensively in the last 50 years (He, 2002) with an enormous introduction of alien plants. There is an urgent need for botanical gardens to devise protocols that minimise invasion risks through risk assessment (e.g. Virtue *et al.*, 2008; Conser *et al.*, 2015) and manipulation of the frequency, magnitude and dispersion of introductions, not only for China, but also worldwide. Indeed, preventing these introductions by botanical gardens or interrupting the gene exchanges between individuals in botanical garden and outside is more cost-effective than eradicating invaders after they have established in the field (Mack *et al.*, 2000). Further, the introduction of plants by botanical gardens can be beneficial for the conservation of plants; yet, plant species conserved in botanical gardens may also be threatened by the plant invasion from surrounding environment. Thus, careful management in botanical gardens can be of significance in weakening the influence of invasive species on the conserved gene pool in botanical gardens.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Line graphs from the admixture model of STRUCTURE of ΔK for ISSR.

Figure S2 Inferred populations structures for the whole sample (701 individuals, 30 sampling populations). This data was used to partition individuals into K = 2, 3, 4, or 5 gene clusters.