



## **Genetic Diversity of *Ambrosia trifida* L. as Revealed by AFLP Markers**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors MS and BQ designed the study. Authors DF and XW performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ZL managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** Invasion of alien species has become a global problem. Many invaders appear to thrive even with low levels of sequence-based genetic variation, and genetic patterns might congruent with the spatial arrangement of populations.

**Study Design:** The genetic diversity of giant ragweed was investigated using amplified fragment length polymorphism (AFLP). There were 5 populations(100 samples), and the distance between the two populations was more than 20 kilometers along with the mainstream of Liaohe.

**Place and Duration of Study:** Samples were collected from Liaohe Basin. Experiments were done in the College of Biological Science and Technology, between February 2016 and March 2017.

**Methodology:** The distance between the two populations was more than 20 kilometers. We sampled five populations of *Ambrosia trifida* L. along Liaohe river in Liaoning province (north-east

of China). Leaves for AFLP experiment were stored in discoloration silica gel. Genomic DNA was extracted following the CTAB protocol.

**Results:** The genetic distances varied with the geographic distances. However, with the influence of many different situations, and many modes of transmission, the discrepancy of genetic distance was reflected. There was low genetic diversity during earlier period of invasion diversity. After a period of invasion, genetic diversity gradually increased. Panshanzha number of polymorphic bands and percentage of polymorphic bands was 98 and 64.47% respectively. Manduhu was 93 and 61.16%, Juliuhu was 100 and 65.79%, Shifosi was 98 and 64.47%, Shuanganqiao was 127 and 83.55%. Shuanganqiao possessed the highest polymorphism.

**Conclusion:** The source of genetic variation of giant ragweed in Liaohe was gene flow of population. Shuanganqiao might be the spread center.

**Keywords:** *Ambrosia trifida* L.; genetic diversity; geographic distance; Liaohe basin.

## 1. INTRODUCTION

Riparian habitat is mainly viewed as a one-dimensional or linear system compared with other habitats with propagules and gametes keeping connectivity of upstream and downstream populations [1-3]. The seeds of riparian and aquatic species along stream gets accumulated at downstream, but few plants live downstream. If upstream species transferred to downstream, that means it is in accordance with the "Drift paradox" theory [4,5]. Therefore, the phenomenon has been termed "unidirectional diversity hypothesis" that downstream genetic diversity increase effectively because of the movement of seeds and propagules from upstream to downstream [4,6-8]. Nevertheless, no clear evidence from previous studies can be proofed this assumption [2,9]. The Liaohe main stream we researched is in Liaoning province about 480 kilometers and the catchment area is about 6.6 thousands square kilometers. The whole landscape pattern tended to be fragmented. The main types were returning farmland to forest, so the health assessment of aquatic ecosystem was not optimistic because of human disturbance [10-12].

Biological invasions imperil biodiversity by altering the structure and functioning of ecosystems. The invaders can change ecological interactions, disrupt mutualistic plant-animal interactions and competition for space, light, soil nutrients or water inducing extinction of native species [13]. As successfully invasive species, we suggested that rapid evolution occurred with high hereditary variation that the gene might be reshuffled and recombined [14-16]. Afterwards, genetic diversity determining genotype diversity can not only increase biomass, nutrient uptake and resist plant invasions but promote resilience from

disturbance and decrease pathogen infection capacity [17-20]. Adaptive evolution studies in invasive species predicted that the neutral or deleterious alleles from standing genetic variation are more beneficial to adaptability and fast evolution than favourable alleles and recessive modifier genes might play an important role [15,21]. Other hypothesis stated the increasing growth rate and vegetative vigor making it extremely aggressive and abundance due to escape of their natural enemies [22] and reallocation of nitrogen (N) and photosynthetic (N) use efficiency make big difference between invader and natives [23-26] showing their higher invasive nature.

Previous studies have shown that the genetic differentiation or variation captured by genetic diversity at neutral sites will increase with the increase of geographical distance [4,6]. The characteristic heterozygosity which is always measured by parameters such as the number of allelic richness can reflect genetic diversity in every population [27,28]. A large number of gene loci are accompanied by the two or more alleles, which display the polymorphism of genetic structure, have the formation of the ecotype and biotype. A population with high genetic diversity means it possesses abundant genetic variation, the powerful adaptability and evolutionary potential. The genetic drift, gene flow, natural selection, gene mutation of the intrinsic factors, and even isolation, environment, human activities the external factors could have an impact on genetic diversity [27,29,30].

*Ambrosia trifida* L. (Giant ragweed), a kind of pernicious weed, was introduced in North China in the 1940s approximately [31]. It has the traits of tall plants and great seed bank, making it disperse to all around up to now. In addition, the pollens of *A. trifida* L. can cause allergic disease

such as the Pollinosis (hay fever) and seasonal allergic rhinitis [32]. In this work, we captured five populations of *A. trifida* L. in Shuangqiao, Shifosi, Juluhe, Manduhu, Panshanzha, along Liaohe, in Northeast of China have been collected and performed the relationship among the 5 populations of ragweed in Liaoning using amplified fragment length polymorphism (AFLP) markers have been studied. We could calculate the genetic distance by the geographical distance, expecting to get the result that genetic distance was proportional to geographical distance, and we could understand that genetic distance was related to geographical distance, traffic condition, or other factors. Finally, we could draw a map of the kinship of all ethnic groups. At present, dominant markers and amplified fragment length polymorphism (AFLP) are considered to be the most valuable methods for evaluating diversity and differentiation, which is based on selective amplification of restricted fragments derived from digestion of the total genomic DNA. It has higher superiority than RFLP and RAPD [33,34].

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

The strategies of sampling methods followed Mark shell and Brown that the distance between

two populations must exceed twenty kilometers and two strains of one group was one meter. We sampled five populations of *Ambrosia trifida* L. along Liaohe river in Liaoning province (north-east of China) (Fig. 1). The sites were mapped using the GPS following the Table 1.

Healthy and insect-free leaves were collected for AFLP analysis, which were preserved in discolored silica gel. Genomic DNA was extracted following the CTAB protocol [35,36]. After the testing, the samples were kept in TE and transported to the laboratory storing at -80°C.

### 2.2 AFLP Analysis

The AFLP analysis was followed the original protocol by Vos et al. (1995). For the first step, genomic DNA was digested with the restriction enzymes *EcoR1* and *Mse1*, then adaptors were linked between ends of genomic fragments and a two-step selective amplification, after that, the pre-selective amplifications and selective amplifications were performed in the specific sample volume, respectively. PCR products were assayed on polyacrylamide gels electrophoresis and stained with silver. Displayed bands were scored as present (1) or absent (0) by visual inspection.

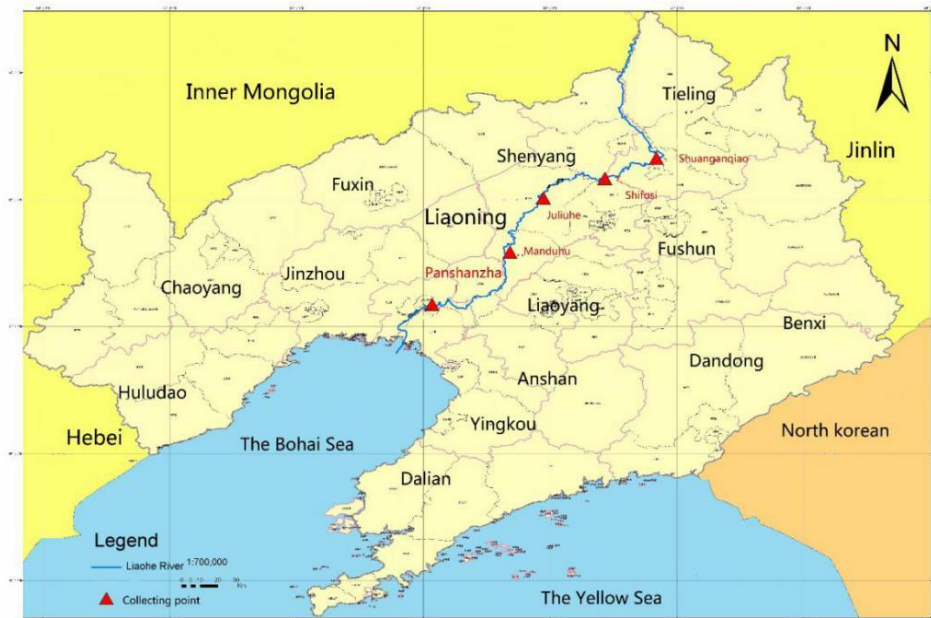


Fig. 1. The AFLP population distribution of giant ragweed in Liaohe River Reserve, Liaoning province, China

**Table 1. The population position basic information of giant ragweed of Liaohe River Reserve, Liaoning province, China**

Name of population	Geographic coordinates	Administrative division
Shuanganqiao	42°19'29"N,123°49'57"E	Yinzhou district, Tieling city, Liaoning
Shifosi	42°09'55"N,123°38'15"E	Cainiu village, Tieling city, Liaoning
Juliuhe	42°00'22.52"N,122°57'50.72"E	Xinmin district, Shenyang city, Liaoning
Manduhu	41°35'17.55"N,122°41'17.19"E	Liaozhong district, Shenyang city, Liaoning
Panshanzha	41°11'11"N,122°05'03"E	Shuangtaizi district, Panjin city, Liaoning

### 3. RESULTS

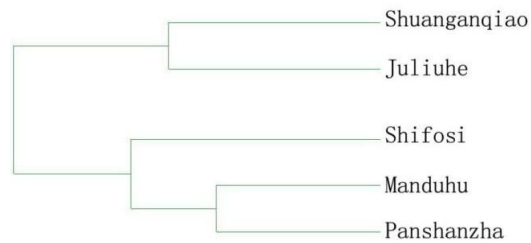
The four pairs of primers for AFLP were selected (E-ACA, M-CTT, E-AGC, M-CTG, E-AGG, M-CAC, E-AGC, M-CAT) by prescreening, with higher polymorphisms, a total of 152 bands was generated and the total bands for 45, 40, 35, 32, the polymorphic bands (PB) values were 36, 28, 29, 26, and the percentages of polymorphic bands (PPB) values were 80%, 70%, 82%, 81% of four primers, respectively (Table 2).

Genetic diversity indices among populations were calculated including the percentage of polymorphic bands (PPB), the effective number of alleles per locus ( $n_e$ ), the observed number of allele per locus ( $n_a$ ), Nei's gene diversity ( $H$ ) and the Shannon's information index of diversity ( $I$ ). The AFLP analysis showed a high level of polymorphism. Among all the population sites, all index number of Shuanganqiao showed the highest value. The PPB value at the population level ranged from 61.18% in population Manduhu to 83.55% in population Shuanganqiao, the mean was 67.48%. The coefficient of genetic differentiation ( $G_{ST}$ ) assessed by Nei's gene diversity among populations was 0.2373, the gene flow ( $H_m$ ) was 1.6071 (Table 3).

Nei's genetic distances and geographic distances between all populations are shown in Table 4. We observed that the genetic distances vary with the geographic distance generally. For instance, the geographic distances from Juliuhe

to Manduhu and Panshanzha were 0.0785 and 0.0865, and the genetic distances were 0.9245 and 0.9171. The minimum genetic distance of two populations, Manduhu and Panshanzha 66 km apart, was 0.0449. The nearest geographic distance of two populations, Shuanganqiao and Shifosi 24 km apart, the genetic distance was 0.1135. However, the results showed that the Shuanganqiao and Juliuhe were abnormal.

The dendrogram of the five populations was demonstrated with the UPGMA method assessed with Nei's genetic distance values and divided the five populations into two branches, Shuanganqiao and Juliuhe were in one branch, and Shifosi, Manduhu and Panshanzha were in another branch (Fig. 2).



**Fig. 2. UPGMA dendrogram showing genetic relationship of the Giant ragweed populations**

Note: The dendrogram of the 5 populations was demonstrated with the UPGMA method assessed with Nei's genetic distance values

**Table 2. Numbers of AFLPs detected with 4 primer pairs for giant ragweed**

Primer	Percentage of polymorphic bands	Number of polymorphic bands	Total number of bands
E-ACA, M-CTT	80%	36	45
E-AGC, M-CTG	70%	28	40
E-AGG, M-CAC	82%	29	35
E-AGC, M-CAT	81%	26	32
Sum	78%	119	152
Mean	78%	29.75	38

**Table 3. Genetic diversity estimates of giant ragweed based on AFLP data**

Population name	Percentage of polymorphic site(a)	Polymorphic sites(b)	Observed alleles(Na)	Effective number of alleles(Ne)	Gene diversity index(Nei)	Shannon(I)	GST	Hm
Shuanganqiao	83.55	127	1.8355	1.4402	0.2643	0.4028	--	--
Shifosi	64.47	98	1.6447	1.3538	0.2077	0.3133	--	--
Juliuhe	65.79	100	1.6579	1.3365	0.1979	0.301	--	--
Manduhu	61.18	93	1.6118	1.331	0.1952	0.2958	--	--
Panshanzha	64.47	98	1.6447	1.3364	0.2019	0.3082	--	--
Mean	67.48	100	1.6567	1.3481	0.2066	0.3024	--	--
Species level	94.74	144	1.9474	1.4446	0.2709	0.4189	0.2373	1.607

**Table 4. Nei's unbiased measures of genetic identity and genetic distance**

Population	Shuanganqiao	Shifosi	Juliuhe	Manduhu	Panshanzha
Shuanganqiao	****	0.8927	0.9357	0.8915	0.9004
Shifosi	0.1135	****	0.9227	0.9215	0.9032
Juliuhe	0.0664	0.0804	****	0.9245	0.9171
Manduhu	0.1148	0.0817	0.0785	****	0.9561
Panshanzha	0.1049	0.1018	0.0865	0.0449	****

The Nei's unbiased genetic distances (above diagonal) and geographic distances (km) (below diagonal) among eight populations of *Ambrosia trifida* L.

## 4. DISCUSSION

### 4.1 Genetic Diversity and Geographic Distance

Considering the AFLP results in 100 plants from five populations, the total of 152 distinct and discernible bands were obtained using 4 pairs of markers and the average pleomorphic bands were 100, 67.48 percent, the species level was 144, 94.74 percent. The Hm index of gene flow between populations was 1.607 indicating that there was a certain gene exchange among the populations of *A. trifida*. The genetic differentiation coefficient ( $G_{st}=0.23$ ) between populations indicated that genetic differentiation among populations was very small, which also indicated that there was a certain gene exchange among populations [37-41]. The geographical distance between Shuanganqiao and Shifosi is 24km, but their genetic differentiation coefficient is 0.6447, which is less than that of the third population of Juliuhe (The genetic differentiation coefficient of the Juliuhe is 0.6579). We combined the actual geographical situation that two sites were separated by Liaohe, traffic jam, and the results indicated that railway line and transportation might be the barriers making the populations isolated [1,6,42]. In this way, we could not find proof to support the "unidirectional diversity hypothesis" that seed and propagule dispersal resulted in accumulation of genetic diversity downstream [4,6,7]. There is a developed transportation between Manduhu and Panshanzha with a genetic distance of 0.0449. The population of Juliuhe and Shuanganqiao are clustered on the same branch, and they are geographically close. There are important national or provincial highways such as 101 National Road and 304 National Road between them, and the traffic is very smooth and developed, which may also be due to the use of means of transportation as a medium of communication. From the history of population development, the fruit of the original same population forms new population in different regions, which may belong to population subdivision [6,43]. At the same time, the existence of gene exchange owing to traffic network between Shuanganqiao and Panshanzha was possible, despite of the far distance. The other branch Shifosi, Manduhu, Panshanzha had lower genetic diversity than Shuanganqiao and Juliuhe. The result concluded that the three populations were corresponded to the theory of founder effect [37,44]. At the beginning of the invasion, with the increase of

the quantity, having no mating with other communities and the difference of each other is tiny. This is because of the impact of the founder effect or genetic bottleneck [16,37], giving rise to the genetic diversity loss in some period. But after a time, the genetic diversity will increase as the gene flow.

### 4.2 Prevention and Control of Invasive Plants

Exotic species, becoming a global problem, can disrupt food chains, transmit disease, reduce biodiversity, and wreak havoc upon agriculture. Biodiversity is one of the most important attributes and a measure of restoration success. However, *Ambrosia trifida* L., since discovered in 1930s in China, is spreading all over the country. Liaoning province is the worst, especially Shenyang and Tieling city. Now Liaohe as the first conservation area to watershed protection object is overgrown with giant ragweed, which reduces biodiversity and makes the ecological management of conservation areas into a bottleneck. In this work, the ragweed spread outwards with the center of Shuanganqiao in Tieling city and its genetic diversity reduced gradually. We discussed the genetic diversity of *Ambrosia trifida* L. grown along Liaohe by AFLP, was to be a better understanding of evolutionary mechanisms of genetic diversity of *Ambrosia trifida* L., as the genetic diversity of giant ragweed had substantial impact on the local habitat. When the genetic distance and geographic distance of ragweed in Liaohe conservation area is cleared, we will obtain the contributory factors of the genetic diversity. Now the most important factor is the transportation and anthropogenic activity. Thus, the protection of the Liaohe region will be achieved [27,42, 45,46].

## 5. CONCLUSION

The genetic distances varied with the geographic distances, yet, with the influence of many different situations, and many modes of transmission, the discrepancy of genetic distance was reflected. There was low genetic diversity during earlier invasion period diversity. After a period of invasion, genetic diversity gradually increased. Panshanzha number of polymorphic bands and percentage of polymorphic bands was 98 and 64.47%. Manduhu was 93 and 61.16%, Juliuhe was 100 and 65.79%, Shifosi was 98 and 64.47%, Shuanganqiao was 127 and 83.55%. Shuanganqiao possessed the highest

polymorphism. So Shuanganjiao might be the spread center. The source of genetic variation of giant ragweed in Liaohe was gene flow of population.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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