academicJournals

Vol. 9(8), pp. 249-256, 30 April, 2014
DOI: 10.5897/SRE2014.5817
Article Number: C543F4644199
ISSN 1992-2248 © 2014

http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Genetic diversity of *Quercus liaotungensis* Koidz populations at different altitudes

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Received 23 January, 2014; Accepted 27 March, 2014

To estimate genetic diversity and genetic structures of *Q. liaotungensis* at different altitudes in Xingtangsi, eight natural populations were surveyed by sequence-related amplified polymorphism (SRAP) markers. A total of 179 bands were amplified by 12 pairs of SRAP primer combinations. The average number of amplification band for each pair primers was 14.9. The percentage polymorphic band (*PPB*) of *Q. liaotungensis* was 100%, Nei's gene diversity (*H*) and Shannon information index (*I*) was 0.3482 and 0.5264 respectively, which indicated the high genetic diversity occurred in *Q. liaotungensis* populations. The highest genetic diversity harbored in population 6, while the lowest in population 4. The genetic diversity of all eight studied populations showed a low-high-low variation pattern along elevation gradients. Analysis of molecular variance (AMOVA) explored that the genetic variation mainly existed within populations (80%) and only 20% of genetic variation between populations of *Q. liaotungensis* ($p<0.001$). STRUCTURE and Principal coordinate analysis (PCoA) further confirmed the AMOVA analysis. Based on the genetic distance between populations, eight populations were mainly clustered into two groups. *Q. liaotungensis'* intrinsic biological characteristics, effective gene flow and microenvironmental heteroplasmy resulted in the genetic distribution of *Q. liaotungensis* populations in Xingtangsi.

Key words: Genetic diversity, gene flow, *Q. liaotungensis*, sequence-related amplified polymorphism (SRAP), Xingtangsi.

INTRODUCTION

Genetic diversity is the product of long-term evolution of a species (Xiao, 2003). It plays an important role in biological diversity, and lays a foundation for the ecological system's diversity and species diversity. The high or low genetic diversity of a species reflects its ability to adapt to the environment changes, and the level of the diversity directly affects the survival and potential evolution of species (Ohsawa and Ide, 2008; Kurt et al., 2011). Genetic diversity can significantly change with the variation of altitudes that regulate ecological conditions in a particular habitat of the plant (Erich and Johann, 2002; Semang et al., 2003; Feng et al., 2004; Ohsawa et al., 2008; Kurt et al., 2011). Consequently, a change pattern of genetic diversity in plant populations along an altitude

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for theoretical interests, utilization and conservation altitude can cause a drastic change in environmental gradient becomes an increasingly attractive subject both Williams, 1979; Baur and Raboud, 1988; Agar et practices because sometimes a relatively small change in al.,2012).Now, some studies have shown that the level of conditions, such as temperature and moisture (Heath and genetic diversity of plant populations decreases or increases along altitudinal gradients (Premoli, 2003).However, there are also researches indicating opposite results, the genetic differentiation within populations fluctuate to a large extent (Li et al., 1998) or do not correlate (Saenz-Romero and Tapia-Olivares, 2003) with an increase in altitude.

Sequence-related amplified polymorphism (SRAP) is a novel, simple and reliable PCR-based marker system (Abedian et al., 2012; Amar et al., 2011; Liu et al., 2008) that amplifies open reading frames (ORFs) (Li and Quiros, 2001) using specific primer pairs. The forward primers preferentially amplify exonic regions, while the reverse primers preferentially amplify intronic regions and regions with promoters. Compared with other molecular markers, SRAP is simple, reproducible, has a reasonable throughput rate, and can be used for different materials, according to its unique primer design (Dong et al., 2010; Cai et al., 2011). In recent years, SRAP markers have been used in many areas, such as plant genetic diversity analysis (Zuo et al., 2009; Zhang et al., 2011; Qu et al., 2008; Fan et al., 2008), identification of varieties (Gai et al., 2011; Li et al., 2006; Zheng et al., 2009), genetic mapping and gene localization (Wang et al., 2007a).

Q. liaotungensis, being a deciduous broad-leaved tree, belongs to *Quercus* of Fagaceae*,* and widely distributes in the northeast and north of China and Inner Mongolia provinces (regions) (Jia et al., 2009). It is wind-pollinated plant, has widely ecological amplitude, can grow on drought, infertility, warm and humid surroundings. Its reproduction is strong, contains more seeding and young seedling understory. The nuts of *Q. liaotungensis* are eaten by some animals because of rich nutrition, high content of nitrogen-free extract, and little crude fiber (Li, 2005). By SSR markers, the genetic diversity of natural *Q. liaotungensis* populations was at a higher level in Shanxi Province, and the majority of genetic variation occurred within populations (Qin et al., 2012). *Q. liaotungensis* populations at Dongling Mountain region also have very high genetic variability, the diversity in the central population was higher than that of the marginal one (Yun et al., 1998). Within its habitats in Xingtangsi, *Q. liaotungensis* plays a very important role in preventing soil erosion and water loss, in regulating microclimate, and also in maintaining ecological stability in general. However, the pattern of genetic variation present in the natural populations of *Q. liaotungensis* along altitudinal gradients is little known. In this study, using SRAP markers to analyze the genetic diversity of *Q. liaotungensis* populations at different altitudes, explore the change pattern of genetic diversity along elevation gradients, and further reveal the factors affected the genetic diversity and structure of *Q. liaotungensis* in Xingtangsi.

MATERIALS AND METHODS

Plant sampling

A total of eight wild populations of *Q. liaotungensis* were sampled along an altitude gradient – 1300, 1400, 1500, 1600, 1700, 1800, 1900, and 2000 m above sea level in Xingtangsi of Huozhou, Shanxi province. Those studied populations marked as population 1 (1300 m), 2 (1400 m), 3 (1500 m), 4 (1600), 5 m (1700 m), 6 (1800 m), 7 (1900 m), 8 (2000 m) respectively. Within each population, 10 to 20 individuals were randomly collected. To avoid collecting the same clone, each sampled individual from the same population was collected from different locations about 30 to 50 m apart. Fresh leaves were collected and immediately stored in liquid nitrogen for genomic DNA extraction.

DNA extraction and PCR amplification

Genomic DNA was extracted using the modified 2×CTAB method (Li et al., 2009). The quality and concentration of the extracted DNA was determined using a UV visible spectrophotometer (Biomate, Thermo Spectronic, Cambridge, UK) and by electrophoresis in 0.8% agarose gels. The DNA samples were diluted to a concentration of 30 ng·μL^{-1} for PCR amplification. DNA samples were stored at -20°C prior to SRAP analysis.

SRAP primers were synthesized by Xi'an Kehao Bioengineering Limited Liability Company. An individual was randomly selected from each population of *Q. liaotungensis* to screen the suitable primer combinations from 88 primer combinations of SRAP. Of these 88 SRAP primer pairs, 12 primer combinations (Table 1) with clear amplification bands and good repeatability were selected to amplify all individuals of eight populations.

The reaction system PCR amplification was optimized in 10 μl, the annealing temperature and PCR amplification cycles of different pair primers were optimized. Within 10 μl reaction system contained: 0.4 μl primers (0.2 μl Me + 0.2 μl Em), 1 μl genomic DNA, 5 μl Mix, and 3.6 μl ddH_2O.

All PCR reactions were performed in a PTC-100 PCR programmable Thermal Controller in the following steps: Pre-denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 35°C for 1 min, extension at 72°C for 90 s, 10 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, 35 cycles of denaturation at 94°C for 1 min, and finally extension at 72°C for 10 min.

The amplification product was detected by 12%(W/V) denatured polyacrylamide gel electrophoresis running at 200 V constant voltage for 200 min and then silver stained according to previously reported procedures (Liu et al., 2008).

Data analysis

As the SRAP marker is dominant, we assumed that each band represented the phenotype at a single biallelic locus. All clearly detectable amplified fragments were scored as either present (1) or absent (0), and a matrix of SRAP data was assembled.

The parameters of genetic diversity: observed number of alleles per locus (*Na*), effective number of alleles for per locus (*Ne*), the percentage of polymorphic bands (*PPB*), Shannon information index (*I*), and Nei's gene diversity(*H*) were estimated by using POPGENE (version 1.31) (Yeh et al., 1997) software. The coefficient of gene differentiation (*G*st) and the level of gene flow (*N*m) were also

Table 1. SRAP primers sequences in this study.

Forward	Sequence(5'-3')	Reverse	Sequence(3'-5')
Me1	TGAGTCCAAACCGGATA	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em6	GACTGCGTACGAATTGCA
Me5	TGAGTCCAAACCGGAAG	Em8	GACTGCGTACGAATTCTG
Me6	TGAGTCCAAACCGGTAA	Em9	GACTGCGTACGAATTCGA
Me7	TGAGTCCAAACCGGTCC	Em10	GACTGCGTACGAATTCAG
		Em11	GACTGCGTACGAATTCCA

Table 2. Genetic diversity of *Q. liaotungensis* populations at different altitudes.

Population	*Na*	*Ne*	*H*	*I*	*PPB*（%）	H_T	*Hs*	G_{ST}	*Nm*
1	1.9162	1.4798	0.2896	0.4428	91.62				
2	1.8771	1.4536	0.2802	0.4297	87.71				
3	1.8324	1.5049	0.2917	0.4353	83.24				
4	1.7542	1.4579	0.2632	0.3927	75.42				
5	1.8045	1.4705	0.2736	0.4103	80.45				
6	1.9050	1.5748	0.3312	0.4917	90.50				
7	1.8883	1.4454	0.2735	0.4217	88.83				
8	1.8939	1.4350	0.2689	0.4159	89.39				
Average	1.8590	1.4778	0.2840	0.4300	85.90				
Species level	2.0000	1.5738	0.3482	0.5264	100.00	0.3482	0.2840	0.1844	2.2108

Na=observed number of alleles; *Ne*=effective number of alleles; *H*=Nei's gene diversity; *I*=Shannon information index; *PPB*=The percentage of polymorphic band; *N*m=estimate of gene flow from *G*st; *H*s=gene diversity within populations; H_T=total gene diversity; *G*st=coefficient of gene differentiation.

measured by using POPGENE. Based on the genetic distance among populations, the dendrogram of populations were made using MEAG (version 5.0) software.

The analysis of molecular variance (AMOVA) was employed to estimate within- and among-population diversity using GenAlEx version 6.0 (Peakall et al., 2006). To further examine the genetic relationships among the populations, the principal coordinate analysis (PCoA) was also used by GenAlEx.

Population structure was analyzed using the software package STRUCTURE version 2.2 (Pritchard et al., 2000; Falush et al., 2003, 2007). The calculation was carried out under an admixture ancestry model and correlated allele frequency model. A burn-in period of 10,000 generations, followed by 50,000 iterations, was used to cluster the population. The assumed number of populations (*K*) was set from 2 to 8. The Δ*K* statistic, based on the rate of change of log likelihood of data [*I* (*K*)] between successive *K* values, was used to select the optimal *K*, following Evanno et al. (2005).

RESULTS

Genetic diversity of *Q. liaotungensis* populations

A total of 179 clear and highly polymorphic bands were detected by 12 SRAP primer combinations. The length of amplified bands was from 125 to 2000 bp. The number of polymorphic bands for per primer combination ranged from 8 to 21, with an average of 14.9.

High genetic diversity in populations of *Q. liaotungensis* was detected by SRAP (Table 2). At the species level, the percentage of polymorphic bands (*PPB)* was 100%, observed number of alleles (*Na*) was 2.0000, effective number of alleles (*Ne*) was 1.5738, Shannon diversity index (*I*) was 0.5264 and Nei's gene diversity index (*H*) was 0.3482 (Table 2). Within each population, the percentage of polymorphic band (*PPB*) ranged from 75.42 to 91.62%, with an average of 85.90%. Shannon information index (*I*) ranged from 0.3927 to 0.4917; Nei's gene diversity (*H*) showed the similar trends, ranging from 0.2632 to 0.3312. Observed number of alleles (*N*a) ranged from 1.7542 to 1.9050. Effective number of alleles ranged from 1.4579 to 1.5748.

At the population level, the average genetic diversity of *Q. liaotungensis* populations was: *PPB*=85.90%, *Na*=1.8590, *Ne*=1.4778, *I*=0.2840, *H*=0.4300 (Table 2). The genetic diversity of population 6 was the highest among all the populations involved in this study (*PPB*: 90.50%, *Na*: 1.9050, *Ne*: 1.5748, *H*: 0.3312, *I*: 0.4917), while the genetic diversity of population 4 was the lowest (*PPB*: 75.42%, *Na*: 1.7542, *Ne*: 1.4579, *H*: 0.2632, *I*: 0.3927). The genetic diversity of all studied *Q. liaotungensis* populations was different along an altitude gradient. As can be seen from Table 2, the genetic

Table 3. Analysis of molecular variance (AMOVA) within/among *Q. liaotungensis* populations.

Source of variance	d.f	SSD	MSD	Ratio of variance (%)
Among populations	7	767.150	109.593	20
Within populations	72	2300.000	31.944	80
Total	79	3 067.150		100

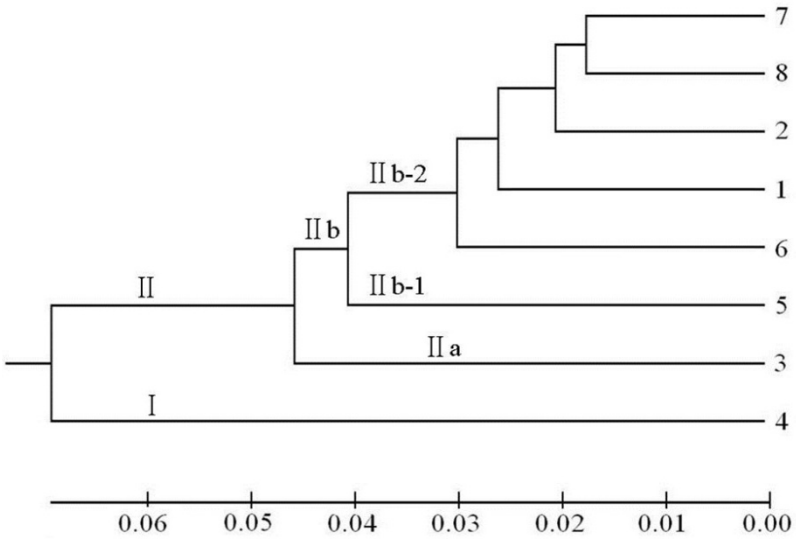

Figure 1. Dendrogram of *Q. liaotungensis* populations based on genetic distance.

diversity of *Q. liaotungensis* populations showed the low-high-low variation pattern with elevation increase. The highest level of genetic diversity was present at an altitude of 1800 m, and following the altitude of 1500 m.

Genetic structure of *Q. liaotungensis* populations

The Nei's gene diversity index always was regarded as one parameter to estimate the genetic differentiation of plant populations (Qin et al., 2010). The average gene diversity (H_S) was 0.2840 within populations of *Q. liaotungensis*. And the coefficient of genetic differentiation among populations (*G*st) was 0.1844 (Table 2). Those indexes revealed that the genetic variance mainly occurred among individuals within populations for *Q. liaotungensis*.

AMOVA (Table 3) showed that there was a significant (p<0.001) genetic difference within eight populations of *Q. liaotungensis*. Of the total genetic diversity, there were 31.978 variance components among populations and 7.265 within populations. The 80% of total variation was contributed by individuals within populations, and 20% occurred among populations. AMOVA result was consistent with the coefficient of genetic differentiation *G*st.

In addition, the effective gene flow per generation for *Q. liaotungensis* was *N*m=2.2108, which was higher than that of the widespread species (*N*m=1.881, Hamrick, 1987).

Genetic distances among populations ranged from 0.0354 to 0.1535 (data not shown), with an average of 0.0873. The genetic identities among populations varied from 0.8577 to 0.9652, with an average of 0.9170. The longest distance occurred between population 7 and population 8, while the shortest was between population 1 and population 4.

The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis based on the genetic distance among populations revealed that all eight populations of *Q. liaotungensis* were separated into two groups (I-II) (Figure 1). Group I included only population 4, while the other populations clustered around Group II. Within Group II, the population 3 clustered around the subgroup IIa, the population 5 clustered around the subgroup IIb-1; other populations gathered around the subgroup IIb-2.

Principal coordinate analysis (PCoA) plot revealed a similar grouping of populations with the UPGMA dendrogram (Figure 2). The first three eigen vectors accounted for 80.10% of the variation observed. In the two-dimensional PCoA, the first two coordinates explained 62.26% (42.08% for axis 1 and 20.18% for axis 2,

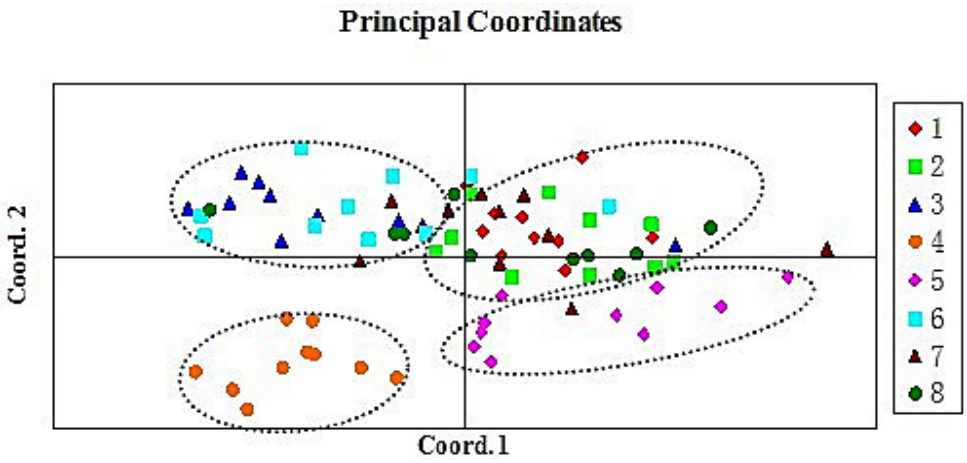

Figure 2. Principal coordinates analysis (PCoA) for *Q. liaotungensis* individuals.

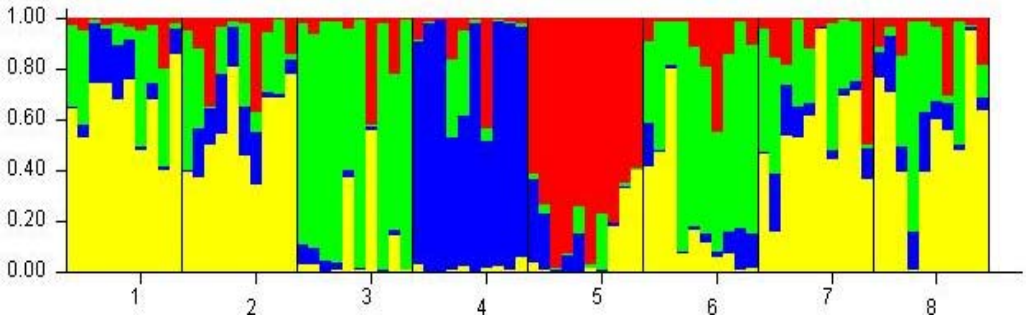

Figure 3. Estimated genetic structure for *K*=4 obtained with the STRUCTURE program for 8 populations of *Q. liaotungensis.*

respectively) of the total variance. The studied eight populations of *Q. liaotungensis* were generally divided into 4 groups. The population 4 alone gathered a group, population 5 gathered a group, population 3 and 6 gathered together, population 1, 2, 7 and 8 gathered together (Figure 2).

The pattern of genetic diversity and structure was further analyzed with a Bayesian-based approach implemented in the program STRUCTURE. The obvious optimum for the ad hoc quantity based on the second order rate of change of the likelihood function with respect to Δ*K* was observed for *K*=4. As a result, the entire populations were successfully assigned to four subgroups (Figure 3). This result was consistent with the PCoA analysis.

DISCUSSION

The genetic diversity of a species can be affected by various factors (Xie et al., 2010; Wang et al., 2007b; Jiang et al., 2009), such as life patterns, breeding system, geographical distribution, mechanism of seed dispersal (Xie et al., 2007, He et al., 2007).

In this study, there existed high genetic diversity in *Q. liaotungensis* populations at Xingtangsi (*PPB*=100%) (Table 2). Compared with other species of Fagaceae, the level of genetic diversity of *Q. liaotungensis* populations at Xingtangsi was higher than that of *Q. mongolica* populations located at Daqinggou (*PPB*=71.7%) and *Q. liaotungensis* populations at Donglingshan (*PPB*=67.6%) (Yun et al., 1998), similar to that of *Q. mongolica* populations (*PPB*=96.8%) (Li et al., 2003).

High genetic diversity of *Q. liaotungensis* populations may have a correlation with the biological characteristics and evolutionary history of this species. *Q. liaotungensis* is an old monoecism plant with unisexual and wind pollination, whose ancestor might have a rich genetic basis that is widely preserved in the long-time evolution. Meanwhile, *Q. liaotungensis*, as a kind of perennial species, can conserve its genetic diversity in quite a long time. Field investigations had found that the survival rate of seedlings of *Q. liaotungensis* in the natural forest was high at Xingtangsi. There were more seedlings and saplings for *Q. liaotungensis,* whose age structure was an ascending pattern. Based on those findings, we can infer

that there existed a high genetic diversity basis for *Q. liaotungensis* in natural forest. In addition, *Q. liaotungensis* can grow in different habitats dying to its intrinsic biological properties, such as strong adaptability, wide ecological amplitude, drought and barren resistance. The heterogeneous environment could promote local adaptation and fixation of different alleles in *Q. liaotungensis*, which also make a contribution to its high genetic diversity.

Among eight populations, the genetic diversity of population 4 was the lowest (*PPB*=75.42%, *I*=0.3927, *H*=0.2632, Table 2), followed by the population 5 (*PPB*=80.45%, *I*=0.4103, *H*=0.2736) and population 3 (*PPB*=83.24%, *I*=0.4353, *H*=0.2917). Those populations located at the medium altitudes in Xingtangsi. With the development of tourism in Xingtangsi, human activity with an elevation between 1500 and 1700 m was very frequent. Subsequently, the large areas of vegetation destroyed by the people and the ecological environment were relatively serious. Those irreversible changes caused the decrease in genetic diversity of intermediate altitude populations. High-altitude populations 6, 7 and 8, located on the edge of the *Q. liaotungensis* distribution in Xingtangsi, had relatively high genetic diversity (above 1800 m). Generally, the temperature drop is about 0.55 with the elevation increasing to l00 m. The rainfall is increased with the rise of altitude; so, the surrounding of those populations was relatively poor, and the environment selection pressure for those populations was relatively bigger; as a result, these populations' genetic diversity was relatively higher. Under the influence of human activity and livestock grazing, it easily led to genetic drift and made the loss of genetic diversity of the populations at low altitude. However, interestingly, populations 1 and 2 at low altitudes had relatively high genetic diversity. The reasons are as follows: *Q. liaotungensis* is still a kind of wild natural population in Xingtangsi, the community structure that had less human destruction is relatively complete, and that the tree age at all levels saved certain individuals of *Q. liaotungensis*, thus avoiding some adverse effect on genetic diversity of *Q. liaotungensis* populations. Based on field investigation, the rich seedling, good recruitment, and different microhabitat resulted in the high genetic diversity in the population 1 and 2.

If the inhabited elevation of the population was divided into three levels - high, medium and low, there should have four kinds of relationship between the genetic diversity and altitudes: i) The genetic diversity of medium elevation is higher than low and high altitude populations; ii) In the high altitude population, genetic diversity is lower than low altitude populations; iii) In the high altitude population, genetic diversity is higher than low altitude populations; iv) The genetic diversity of populations has no relationship with altitude (Ohsawa and Ide, 2008; Ohsawa et al., 2008). In this paper, the population 6 has the highest genetic diversity at altitude of 1800 m. With the rise of altitude, the level of genetic diversity of *Q. liaotungensis* populations gradually decreased. When the altitude dropped to 1300 m, the level of genetic diversity of *Q. liaotungensis* has a certain degree of increase. Therefore, a close relationship had existed between the elevation and genetic diversities of *Q. liaotungensis* populations. The genetic diversity of *Q. liaotungensis* presents a low-high-low pattern along altitude gradient. Those results supported the viewpoint that the genetic diversity of medium elevation is higher than low and high altitude populations.

AMOVA analysis showed that 80% of total genetic variation of *Q. liaotungensis* was contributed within populations and 20% among populations (p<0.001).

The genetic structure of populations not only depended on breeding system, origin and evolution process, but also affected by natural selection, gene flow and other factors (Peng et al., 2007; Ishihama et al., 2005). High level of gene flow could prevent the genetic differentiation among population, while low level of gene flow might make the population better adapt to the local environment, and increase the genetic isolation among populations. Wright (1931) pointed out that if the gene flow among populations was less than 1, the gene drift would play an important role in influencing genetic variation and genetic structure (Hey et al., 2012). *Q. liaotungensis'* pollen, being small and light, can distribute from high altitude to low altitude. *Q. liaotungensis'* nuts, large volume, can fall to low altitude area because of the gravity. And the nuts' nutrition was rich, which was important food for small mammals and birds. Thus, the seeds flow among different population could occur. Effective flow of pollen and seed made the high level of gene flow among populations of *Q. liaotungensis* (N_m=2.2108). Although the genetic variation mainly existed in *Q. liaotungensis* populations, a significant genetic differentiation still occurred among populations (Table 3, Figures 2 and 3). In a small area, the genetic drift was a main factor that caused the genetic differentiation among populations (Li et al., 2011; Zhang et al., 2010). The microhabitat heterogeneity in the relatively small geographical area also resulted in the genetic differentiation among populations (Li et al., 2004; Zhang et al., 2005; Zhao et al., 2001). With increasing elevation, climate, vegetation and soil showed obvious vertical variation; the temperature, water, light, and other ecological factors were also significantly different in different altitudes for different population of *Q. liaotungensis*, which eventually led to the genetic differentiation that occurs among populations.

Conclusion

Q. liaotungensis populations had high genetic diversity, and the genetic variation mainly existed within populations. There a significant genetic differentiation occurred among populations. The genetic diversity of all studied eight *Q. liaotungensis* presents a low-high-low pattern along

altitude gradient. *Q. liaotungensis'* biology characteristics, effective gene flow and environmental heteroplasmy resulted in the genetic distribution pattern of *Q. liaotungensis* populations in Xingtangsi.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This research was supported by the funding of the undergraduate innovative experiment project of Shanxi Normal University (SD2010XCSY-19), the Natural Science Foundation of Shanxi Normal University (ZR1106) and the Natural Science Foundation of Shanxi Province (2011011031-2).

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