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Genetic variation of hazel (*Corylus heterophylla*) populations at different altitudes in Xingtangsi forest park in Huoshan, Shanxi, China

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Abstract

The change pattern of genetic diversity along an altitude gradient has become an increasingly attractive subject in both theoretical studies and utilization and conservation practices because altitude variations regulate the ecological conditions in a particular plant habitat; sometimes, even a relatively small change in altitude can cause a drastic change in environmental conditions. *Corylus heterophylla*, which grows along an altitude gradient ranging from 1200 m to 2400 m, is an important economic shrub species that helps maintain vegetation stability and ecological equilibrium in Xingtangsi forest park in Huoshan, Shanxi. In this study, eight *C. heterophylla* populations were sampled along an altitude gradient (each sampling interval was about 100m). By using a SRAP molecular marker, the genetic diversity and variation of *C. heterophylla* populations in Xingtangsi forest park were studied. The results showed that the *C. heterophylla* populations had high genetic diversity. The effective number of alleles (*Ne*), Nei's gene diversity index (*H*), and Shannon diversity index (*I*) were 1.5106, 0.3130, and 0.4790, respectively. The total percentage of polymorphic loci (*PPB*) was 98.03%. Of the eight populations, population 3 had the highest genetic diversity（*Na*:1.8465, *Ne*:1.5759, *H*:0.3203, *I*:0.4683, *PPB*:84.65%), whereas population 1 had the lowest (*Na*:1.6102, *Ne*:1.2997, *H*:0.1841, *I*:0.2841, *PPB*:61.02%). The variation in genetic diversity of all eight studied populations indicated a low-high-low pattern along the elevation gradients. The mid-elevation populations (1700m, 1800m, and 2000m) were more genetically diverse than both low-elevation (1500m) and high-elevation populations (2100m and 2200m). AMOVA analysis showed that only 16% of the total genetic variation occurred among populations, whereas 84% of the variance was within populations; this was in line with the coefficient of genetic differentiation (G_{ST} = 0.225) and the STRUCTURE analysis. The cluster analysis (UPGMA) results indicated that eight *C. heterophylla* populations were mainly grouped into 5 clusters; the PCoA results also showed the populations of *C. heterophylla* approximately divided into 5 groups. Based on the available data, it is likely that the biological characteristics, effective gene flow, and environmental heteroplasmy of *C. heterophylla* are enough to significantly differentiate the populations of *C. heterophylla* in Xingtangsi forest park in Huoshan, Shanxi.

Keywords: *Corylus heterophylla*, different altitude, genetic variation, SRAP.
Abbreviations: SRAP_sequence-related amplified polymorphism; CTAB_hexadecyl trimethyl ammonium bromide; AMOVA_ analysis of molecular variance; PCoA_ principal coordinate analysis; UPGMA_unweighted pair-group method with arithmetic means.

Introduction

Corylus heterophylla belongs to the genus *Corylus* of Betulaceae. It is a deciduous and shade-tolerant shrub mainly distributed in forests, forest edges, and the southern slope of mountains (Cheng et al., 2012). *C. heterophylla* has unisexual flowers, is monoecious and wind-pollinated, has a wide ecological amplitude, and can grow in drought, infertile conditions, and warm surroundings. It is an excellent greening plant for soil improvement and soil and water conservation in mountain areas. *C. heterophylla* is regarded as an economically important shrub species because its nuts have a high oil content, its fruit shells are a source of active carbon, and its bark and fruit buds provide tannin extract (Gao, 2012). Most previous studies on this species have focused on its growth habits (Dong and Wang, 2009), cultivation techniques (Li et al., 2010 a), breeding, nursery and field management (Li, 2010), physiology and morphology (Li et al., 2010 b), and chemical components (Tao et al., 2006). Little is known about the genetic diversity and population structure of *C. heterophylla* at different altitudes. An examination of the genetic diversity among populations within a species is crucial for a better understanding of evolutionary processes and the nature of the species. Many plant species grow within a range of different habitats and have developed adaptive strategies suited to their particular habitat (Schneller and Liebst, 2007). Because elevation is a complex factor, altitudinal gradients comprise an assemblage of environmental variables, such as rapidly changing climate conditions, which markedly influence the distribution of population genetic variation of plant species (Chen et al., 2008; Hahn et al., 2012). The potential of altitudinal climatic gradients to influence the distribution of genetic variation within and among plant populations is highly complex and variable among species (Byars et al., 2009; Hahn et al., 2012). Along altitudinal gradients, the genetic differentiation between populations of some plant species results in rapid, elevation-related changes in environmental conditions (Seman et al., 2003; Bellusci et al., 2005; Liu et al., 2006; Zhang et al., 2006; Chen et al., 2008). However, some studies have reported only a little or no differentiation with respect to altitude (Aradhya et al., 1993; Oyama et al., 1993; Gehring and Delph, 1999; Hahn et al., 2012). Due to the uncertainty of plant responses to climate

change, an understanding of current distribution patterns of population genetic diversity and differentiation (McMahon et al., 2011) along altitudinal gradients is fundamental for the availably conservation, reasonable utilization, and establishment of management strategies for resilient species. According to our previous field investigations in Xingtangsi forest park in Huoshan, the growth, morphological characteristics, spatial pattern, and population structure of *C. heterophylla* are closely related to altitudinal gradients, although the changes in these characteristics occur nonlinearly along increasing altitudes (Gao, 2012). Therefore, studies on the level of genetic diversity and its partitioning among *C. heterophylla* populations at varying altitudes in this region are of prime interest. In recent years, many studies on genetic variation along altitudinal ranges have been done with the aid of molecular markers (Chen et al., 2008; Truong et al., 2007; McMahon et al., 2011; Hahn et al., 2012). Sequence-related amplified polymorphism (SRAP) is a novel and PCR-based molecular marker that amplifies open reading frames (ORFs) (Li and Quiros, 2001) by using specific primer pairs. Compared with other molecular markers, SRAP is simple and reproducible, has a reasonable throughput rate, and can be used for different materials according to their unique primer designs (Dong et al., 2010; Cai et al., 2011). SRAP markers have been widely used for germplasm identification (Ferriol et al., 2003; Budak et al., 2004), linkage map construction (Lin et al., 2009), gene tagging and mapping (Qiao et al., 2007), map-based cloning, genomic studies (Sun et al., 2007), cDNA fingerprinting (Liu et al., 2008), and evolutionary and genetic diversity analyses (Amar et al., 2011; Abedian et al., 2012; Dong et al., 2010; Cai et al., 2011). In this study, we use SRAP markers to analyze the genetic variation of *C. heterophylla* populations at different altitudes, explore the change pattern of genetic diversity along elevation gradients, and reveal the factors affecting the genetic variation and population structure in Xingtangsi forest park.

Results

Genetic diversity of C. heterophylla populations

By using the optimized SRAP-PCR amplification system, a total of 768 clear and polymorphic bands were detected by 13 pairs of primer combinations. The length of the amplified bands was from 125 to 2000bp. Among the 768 amplified fragments, 567 (73.87%) were polymorphic, with an average of 59 fragments for each primer combination. The number of polymorphic bands per primer pair ranged from 3 to 8, with a mean of 5.8. The highest Nei's gene diversity *H* and Shannon's information index *I* were detected by the primer pair Me8-Em9, whereas the lowest was detected by Me2-Em1. Table 1 shows the genetic diversity parameters at both the population level and the species level. High genetic diversities were detected in the populations of *C. heterophylla* in Xingtangsi forest park. At the species level, the percentage of polymorphic bands (*PPB)* was 98.03%, the observed number of alleles (*N*a) was 1.9803, the effective number of alleles (*N*e) was 1.5106, the Shannon diversity index (*I*) was 0.4790, and the Nei 's gene diversity index (*H*) was 0.3130 (Table 1). Within each population, the percentage of polymorphic bands (*PPB*) ranged from 61.02% to 84.65%, with an average of 98.03%. The Shannon information index (*I*) ranged from 0.2481 to 0.4683; the Nei's gene diversity index (*H*) showed similar trends, ranging from 0.1841 to 0.3203. The observed number of alleles (*N*a) ranged from 1.6102 to 1.8465. The effective number of alleles ranged from 1.2997 to 1.5759. At the population level, the average genetic diversity of *C. heterophylla* populations was *PPB*: 73.87%, *N*a: 1.7387, *N*e: 1.4142, *I*: 0.3690, and *H*: 0.2445 (Table 1). Among all the populations in this study, population 3 had the highest genetic diversity (*PPB*: 84.65%, *N*a: 1.8465, *N*e: 1.5759, *H*: 0.3203, *I*: 0.4683), whereas population 1 had the lowest (*PPB*: 61.02%, *N*a: 1.6102, *N*e: 1.2997, *H*: 0.1841, *I*: 0.2841). The genetic diversity of all eight populations of *C. heterophylla* differed along an altitude gradient. The level of genetic variation of the eight *C. heterophylla* populations from the highest to the lowest was 3>4>6>2>7>5>8>1. As indicated in Table 1, the genetic diversity index of the eight *C. heterophylla* populations showed a low-high-low variation pattern with elevation increase. The highest level of genetic diversity was at an altitude of 1700 m followed by that at an altitude of 1500 m.

Genetic structure of C. heterophylla populations

An AMOVA of the distance matrix for the all individuals permitted a partitioning of the overall variation into two levels: among species and among populations within a species. The results revealed that a significantly level of genetic differentiation was present among the *C. heterophylla* populations ($P<0.001$). Of the total genetic variability, there were 0.299 variance components among populations and 1.570 within populations. Of the total variation, 16% was due to individuals among populations, and 84% occurred within of *C. heterophylla* populations (Table 2). The total gene diversity (H_T) and the gene diversity within *C. heterophylla* populations (H_S) were 0.4850 and 0.3759, respectively. The genetic differentiation coefficient G_{ST} was 0.225, which indicated that 22.5% of the total genetic variation in the differentiation occurred between populations and 77.5% came from within the populations. This was consistent with the AMOVA results. The level of gene flow (*N*m, the number of migrating individuals among populations per generation) was estimated to be 1.7275.

Genetic relationships among C. heterophylla populations

Based on the genetic distance among populations, the UPGMA cluster analysis assigned the eight populations into two groups (I-II) (Fig. 1). Group I included only population 1. Group II consisted of the seven other populations and could be further divided into two subgroups, IIa and IIb. Subgroup IIa included only population 4, whereas subgroup IIb comprised six populations (2, 3, 5, 6, 7, and 8). Within subgroup IIb, populations 7 and 8 formed cluster IIb-1; the other four populations (2, 3, 5, and 6) were grouped into cluster IIb-2. The PCoA plot (Fig. 2) revealed a similar grouping of populations to the UPGMA dendrogram (Fig. 1). The first three eigenvectors accounted for 88.97% of the variation observed. The first two coordinates explained 78.78% (45.23% for axis 1 and 33.55% for axis 2) of the total variance. As shown in Fig. 2, the 8 populations were divided into 5 groups: population 1 formed one group, population 4 formed another group, and population 6 formed a third group; populations 2, 3, and 5 were grouped together, as were populations 7 and 8 (Fig. 2). The patterns of genetic diversity and structure were further analyzed with a Bayesian-based approach implemented in the program STRUCTURE, version 2.2. The obvious optimum for the ad hoc quantity based on the second-order rate of change of the likelihood function with respect to ΔK was observed for K=5.

Table 1. Parameters of genetic diversity in eight natural populations of *C. heterophylla*.

Population	*Na*	*Ne*	*H*	*I*	*PPB*
1	1.6102	1.2997	0.1841	0.2841	61.02 %
2	1.7717	1.3964	0.2373	0.3628	77.17 %
3	1.8465	1.5759	0.3203	0.4683	84.65 %
4	1.8346	1.4693	0.2777	0.4192	83.46 %
5	1.6850	1.2758	0.1766	0.2807	68.50 %
6	1.7835	1.4555	0.2719	0.4089	78.35 %
7	1.7677	1.4966	0.2861	0.4235	76.77 %
8	1.6102	1.3443	0.2019	0.3046	61.02 %
Mean	1.7387	1.4142	0.2445	0.3690	73.87 %
Species level	1.9803	1.5106	0.3130	0.4790	98.03 %

Na: Observed number of alleles; *Ne*: Effective number of alleles； *H*: Nei's gene diversity index; *I*: Shannon diversity index; *PPB*: Percentage of polymoiphic bands.

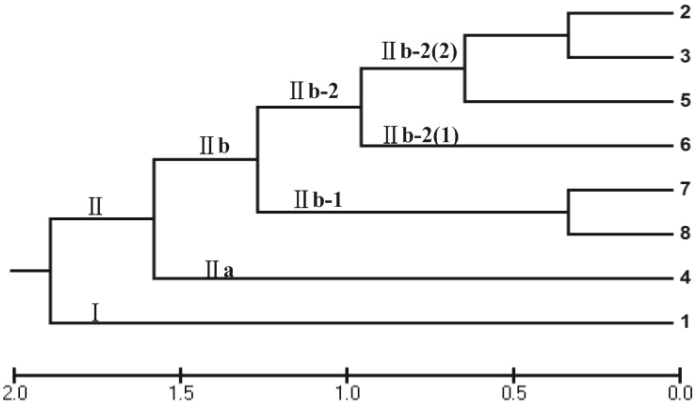

Fig 1. Dendrogram of *C. heterophylla* populations generated using MEAG4 cluster analysis based on genetic distance. Eight populations gathered into I and II two clusters.

As a result, all the populations were successfully assigned to five subgroups (Fig. 3). This result was consistent with that of the PCoA analysis. Regression analysis revealed that a significant positive correlation existed between *Ne* (r=0.0053, p<0.01), *H* (r=0.0046, p<0.01), *I* (r=0.0028, p<0.01), and altitudes, respectively; no significant positive correlation between *Na* (r=0.0126, p> 0.01), *PPB* (r=0.0128, p> 0.01), and altitudes, respectively.

Discussion

Availability of SRAP markers

Compared with other markers, SRAP has several advantages: it is simple and reproducible, has a reasonable throughput rate, provides ease of sequencing of selected bands, and targets open reading frames (ORFs). Budak et al. (2004) compared four marker systems and found the values of revealing genetic diversity power is: SRAP>SSR>ISSR> RAPD. Yang et al. (2012) also found that SRAP markers generated more polymorphic alleles than did SSR markers. Those results were expected due to the high effective multiplex ratio of SRAP (Li and Quiros, 2001).

The results of the present study confirmed that SRAP markers could be powerful tools for detection of genetic diversity among and within species according to 13 pairs of primer combinations. Other studies (Peng et al., 2008; Cai et al., 2011; Huang et al., 2012; Abedian et al., 2012; Yang et al., 2012) have showed that SRAP is more informative, but with a smaller number of SRAP primer combinations. Peng et al. (2008) suggested that SRAP markers could detect a larger number of polymorphisms in a more efficient way with nine SRAP primer combinations. With 15 primer combinations, Huang et al. (2012) found that the SRAP method produced more polymorphic bands per primer, which indicated a high potential for detecting polymorphisms at the species level. Abedian et al. (2012) also believed that SRAP data were informative for the investigation of the genetic diversity of species, with thirteen primer combinations.

Genetic diversity of C. heterophylla populations

In this study, a high level of genetic diversity (as measured by mean *PPB*=98.30% and *I*=0.4790) occurred in eight *C. heterophylla* populations at different altitudes in Xingtangsi forest park (Table 1). The degree of diversity was similar to the values previously reported for *Acer ginnala* (*PPB*=100% and *I*=0.5070) and *Quercus liaotungensis* (*PPB*=100% and *I*=0.5264) in Xingtangsi forest park (Yan et al., 2010). The high genetic diversity in *C. heterophylla* populations may relate to the biological characteristics and living conditions of this species. *C. heterophylla* is an old plant that is unisexual and wind-pollinated, the ancestor of which might have a rich genetic basis that is widely preserved in its longtime evolution. Furthermore, *C. heterophylla*, as a kind of perennial species, can conserve its genetic diversity for quite a long time. Field investigations have found that *C. heterophylla* is still a kind of wild natural population in Xingtangsi forest park, that the community structure that had less artificial destruction was relatively complete, and that the tree age at all levels saved a certain number of trees, thus avoiding some adverse effects on the genetic diversity of *C. heterophylla* populations. Regression analysis showed that a positive correlation existed between genetic diversity indexes

Table 2. Analysis of molecular variance (AMOVA) within / among populations of *C. heterophylla.*

Source of variance	d.f	SSD	MSD	Variance component	Percentage of variance	*P*
Among population	7	36.932	5.276	0.299	16%	<0.010
Within population	92	144,478	1.570	1.570	84%	<0.010

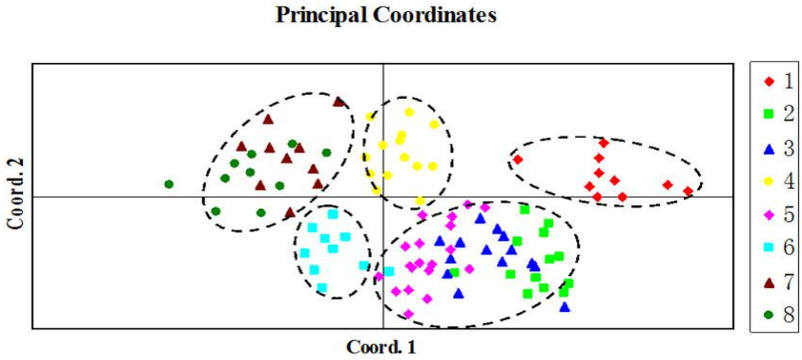

Fig 2. The Principal Coordinates analysis (PCoA) for *C. heterophylla* individuals. Different colors represent the individuals of different populations.

and altitudes. The genetic diversity of all eight studied *C. heterophylla* populations varied significantly with changing elevation, with the trend indicating that mid-elevation populations (1700m, 1800m, and 2000m) were more genetically diverse than both low-elevation (1500m) and high-elevation populations (2100m and 2200m) (Table 1). *C. heterophylla* is believed to be stressed by high temperature at low elevations and by low temperature at high elevations (Li et al., 2007; Chen et al., 2008). Unfavourable environments at both low and high altitudinal zones may lead to an increase in vegetative reproduction and to a decrease in resource-demanding sexuality, which may result in a loss of genetic variation (Chen et al., 2008; Hahn et al., 2012). In addition, from a geographic point of view, the low- and high-elevation populations can be considered marginal. The lower levels of genetic diversity in low-elevation populations (population 1) may also be ascribed to the negative effects of anthropogenic pressures, such as road building and cutting down by humans, which gradually disrupt the habitats of *C. heterophylla* and result in reduced population density and size, and habitat fragmentation. The presence of lower genetic diversity in high-elevation populations (population 8) is likely affected by decreased sexual reproduction imposed by pollination limitation and a shortened flowering season (Young et al., 2002; Hahn et al., 2012). Natural selection in harsh environments may also lead to a loss in genetic variability. Generally, the temperature drop is about 0.55 as the elevation increases by l00m. Rainfall increases with the increase in altitude. Thus, the surroundings of population 8 (2200m) are relatively poor. Under more competitive conditions, individuals showed lower genetic diversity. The high genetic variability present in the mid-elevation populations of *C. heterophylla* is assumed to be related to the position along altitudinal clines. The geographically central populations experience optimal conditions. In the core populations, an effective gene flow and a large population size would weaken genetic drift, thereby increasing genetic variation and reducing the differentiation of population (Hahn et al., 2012). Moreover, better seeding development (germination and growth) has been found in mid-elevation populations compared with those at lower and higher elevations. Relatively large genetic variation can be maintained only in the core populations. Unexpectedly, the genetic variation of population 5 at mid-elevation was the lowest (Table 1). Through a field investigation, we found that due to the development of tourist attractions, there is frequent human activity at an elevation of 1900m, which greatly affected the vegetation. A large area of vegetation had been cleared and the degree of artificial destruction in the environment was relatively serious. This may explain the decrease in genetic diversity of populations at intermediate altitudes.

Genetic structure of C. heterophylla populations

The genetic structure of *C. heterophylla* in Xingtangsi forest park showed a pattern of higher genetic diversity within populations and lesser variation among populations (Table 2). In the present study, the level of genetic differentiation (G_{ST}), recorded for *C. heterophylla* within a geographically restricted area was 0.225. The AMOVA also showed that 16% of the total variability occurred among *C. heterophylla* populations (Table 2); this was confirmed by the UPGMA, PCoA, and STRUCTURE analysis (Figs. 1, 2, and 3). Natural selection, genetic drift, gene flow, mutation, and other factors generally influence the genetic structure of plant populations. *C. heterophylla* is wind-pollinated; thus, its pollen can be distributed from high to low altitudes. In addition, *C. heterophylla* nuts, which are large in volume, can be carried to lower altitudes because of gravity; the nuts are rich in nutrition and serve as an important food for small mammals and birds. Wright (1931) considered level 1 as the critical point in the evaluation of gene flow; thus, if the gene flow among populations was higher than 1, it would play an important role in decreasing the differentiation among populations. In our study, the gene flow of *C. heterophylla* was 1.7275, indicating that the gene flow was an important factor influencing the genetic structure of *C. heterophylla* populations. Gene mutation is often not considered to be a main factor in the differentiation among populations because of its very low occurrence frequency. The neutral theory holds that much of the genetic variation at the molecular level, being neutral or nearly neutral, is almost never affected by the environmental conditions of the species; thus, the genetic

Table 3. Samples information of *C. heterophylla* in the present study.

Populations code	Elevation (m)	Samples	Habitat
1	1500	15	Roadside
2	1600	10	South-facing slope, North-facing slope
3	1700	18	South-facing slope, flat
4	1800	18	South-facing slope, flat
5	1900	20	South-facing slope, flat
6	2000	15	South-facing slope, North-facing slope
7	2100	18	South-facing slope, North-facing slope
8	2200	14	South-facing slope, North-facing slope

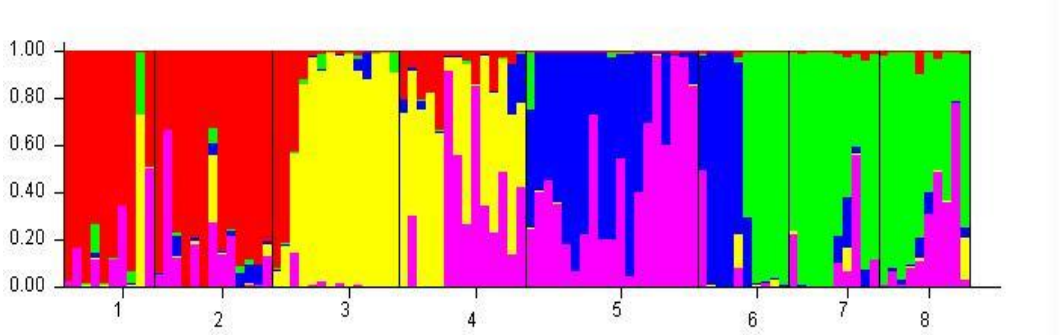

Fig 3. Estimated genetic structure for *K*=5 obtained with STRUCTURE program for 8 populations of *C. heterophylla* based on SRAP variation. The different colours represent different groups.

Table 4. SRAP primers sequences in this study.

Primers	Sequence (5'-3')	Primers	Sequence(3'-5')
Me1-Em1	F: TGAGTCCAAACCGGATA R: GACTGCGTACGAATTAAT	Me4- Em10	F: TGAGTCCAAACCGGACC R: GACTGCGTACGAATTCAG
Me1- Em6	F: TGAGTCCAAACCGGATA R: GACTGCGTACGAATTGCA	Me5- Em8	F: TGAGTCCAAACCGGAAG R: GACTGCGTACGAATTCTG
Me2-Em1	F: TGAGTCCAAACCGGAAT R: GACTGCGTACGAATTAAT	Me7- Em9	F: TGAGTCCAAACCGGTCC R: GACTGCGTACGAATTCGA
Me2- Em8	F: TGAGTCCAAACCGGAAT R: GACTGCGTACGAATTCTG	Me7- Em11	F: TGAGTCCAAACCGGTCC R: GACTGCGTACGAATTCCA
Me3- Em2	F: TGAGTCCAAACCGGAAT R: GACTGCGTACGAATTTGC	Me8- Em9	F: TGAGTCCAAACCGGTGC R: GACTGCGTACGAATTCGA
Me3-Em3	F: TGAGTCCAAACCGGAAT R: GACTGCGTACGAATTGAC	Me8- Em11	F: TGAGTCCAAACCGGTGC R: GACTGCGTACGAATTCCA
Me4- Em6	F: TGAGTCCAAACCGGACC R: GACTGCGTACGAATTGCA		

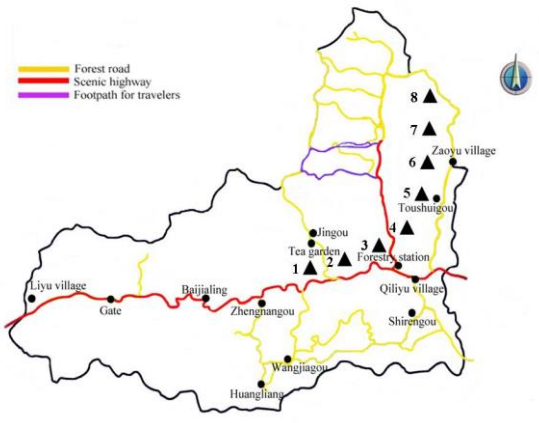

Fig 4. The samples and collections of *C. heterophylla* populations in Xingtangsi forest park in Huoshan, Shanxi. The triangles represent different sample location. Each collection regard as a population.

differentiation among *C. heterophylla* populations was partly caused by genetic drift. The geographic isolation of different environmental conditions might be the major reason for the genetic differentiation among populations of *C. heterophylla*. Variations in elevation, climate, temperature, and soil would result in genetic composition differences among populations. Jin and Li (2005) believed that the genetic variation among populations distributed over limited geographic areas was the result of the pressure of habitat selection. Along an altitudinal increase in Xingtangsi forest park, the climate, vegetation, and soil showed obvious vertical variation. The variation of different populations at different altitudes would result in a shortened life span and delayed pollination phenology for the plant, which would affect the gene flow and selection resulting in genetic differentiation among populations of *C. heterophylla*. The genetic structure along altitudinal gradients may also be influenced by interspecific hybridization. Populations of one species often occur adjacent to closely related species within different altitudinal ranges, coinciding at the boundaries. Consequently, the alleles of some species can flow into other species, causing a genetic cline at particular loci along altitudinal gradients. Such phenomena may also affect the distribution of intrapopulation genetic diversity because the inflow of new alleles can increase allelic richness. The floral asynchrony at different altitudes was another reason for the variation among *C. heterophylla* populations. An increase in altitude causes a decrease in temperature and, further, postpones the flower development. Generally, flowering is delayed by 2-3 days for every 200 m of elevation in Xingtangsi forest park. Therefore, the flowering periods do not overlap in populations or subpopulations with altitude differences greater than 500 m. It follows that the phenological gap also contributes to the observed differentiation. Finally, the genetic distance between populations is an important index for evaluating the level of genetic differentiation among populations of a species (Huang et al., 2008). In this study, the genetic distance between *C. heterophylla* populations was within a wide range, indicating different levels of genetic differentiation for different populations. The genetic distance between populations within a narrow range of altitude was small, whereas that between populations with a greater difference in altitude was relatively large, which showed that a correlation existed between genetic distance and altitude (Huang et al., 2008). The genetic distance between populations 1 and 2 was the smallest, probably due to the similar surroundings and natural selection pressure within a narrow range of altitudes. Similar results were found for *Pinus massoniana*(Li and Peng, 2001), *Psathyrostachys huashanica*(Zhao et al., 2001), *Liquidambar formosana* (Shi et al., 2005), and *Pinus koraiensis* (Feng et al., 2004).

Materials and Methods

Plant materials

During the 2011 growing season, eight populations of *C. heterophylla* were sampled along an altitude gradient at Xingtangsi forest park in Huoshan, Shanxi, China. The sampling locations were selected along a vertical transect that spanned approximately 1400 m. Each sampling interval was about 100m. The altitudinal populations 1-8 (Table 3, Fig. 4) correspond to the altitudes 1500, 1600, 1700, 1800, 1900, 2000, 2100, and 2200m, respectively. Within each population, 10-20 individuals were randomly collected. To avoid collecting the same clone, each sampled individual from the same population was collected from different locations that were about 30m-50m apart. For molecular analysis, fresh leaves were collected from each plant and immediately stored in liquid nitrogen for genomic DNA extraction.

DNA extraction and PCR amplification

Genomic DNA of *C. heterophylla* was extracted by using the modified 2×CTAB method (Wang and Fang, 1998). The purity and concentration of the extracted DNA was determined with a UV-visible spectrophotometer (BioMate; Thermo Spectronic, Cambridge, UK) and by electrophoresis in 1% agarose gel. The DNA samples were diluted to a concentration of 40ng/μl for PCR amplification and stored at -20°C before SRAP analysis. Based on Li and Quiros (2001), the SRAP primers were synthesized at Xi'an Kehao Bioengineering Company. An individual from each population was randomly selected for screening the suitable primer combinations from the 88 pairs of primer combinations of SRAP. Thirteen pairs of primer combinations (Peng et al., 2008; Cai et al., 2011; Huang et al., 2012; Abedian et al., 2012; Yang et al., 2012) with clear amplified bands and good repeatability were selected for amplifying all individuals in the studied populations (Table 4). The PCR amplification reaction system was optimized in 10 μl; the annealing temperature and PCR amplification cycles of different primer pairs were also optimized. The 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 done 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 45s, annealing at 55 to 60 °C for 55s, and extension at 72 °C for 1 min; 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; and 31 cycles of denaturation at 94 °C for 1 min, and finally extension at 72 °C for 7 min. The amplification product was detected with 12%(w/v) denatured polyacrylamide gel electrophoresis ran at a constant voltage of 200 V for 200 min and then it was silver stained according to previously reported procedures (Liu et al., 2008).

Data analysis

All clearly detectable SRAP product bands were scored as either present (1) or absent (0), and a matrix of SRAP data was assembled. Only reproducible and well-defined bands were scored. The parameters of genetic diversity, observed number of alleles per locus (*N*a), effective number of alleles per locus (*N*e), percentage of polymorphic bands (*PPB*), Shannon's information index (*I*), and *N*ei's gene diversity(*H*) were estimated by using POPGENE (version 1.31) (Yeh et al., 1997). The genetic structure was further investigated with the use of Nei's gene diversity statistics, which included the total genetic diversity (H_T), the genetic diversity within populations (H_S), and the relative magnitude of genetic differentiation among populations (G_{ST}= (H_T − H_S)/ H_T) (Nei, 1973). An estimate of the gene flow among populations (*N*m) was computed with the formula *N*m = (1-G_{ST})/2G_{ST} (Mcdermott and Mcdonald, 1993). The analysis of molecular variance (AMOVA) was used to estimate the within- and among-population diversity with the use of GenAlEx version 6.4 (Peakall and Smouse, 2006). To further examine the genetic relationships among the populations, a dendrogram was constructed based on the genetic distance matrix by using the unweighted pair-group method with arithmetic mean s (UPGMA) clustering algorithm. Bootstrap analysis of the UPGMA tree was done with MEGA4 (Tamura et al.,

2007) in 1000 replicates. To obtain an additional representation of the genetic relationships among populations, a principal coordinate analysis (PCoA) from the binary matrix of SRAP was subsequently done with GenAlEx version 6.4. For better understanding of the genetic structure of this species, the population structure was analyzed with 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 a 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 2 to 8. Five independent runs were done for each *K* value. The other parameters were set to default values, as suggested by Pritchard and Wen (2004). The Δ*K* statistic, based on the rate of change in the log likelihood of data [*l* (*K*)] between successive *K* values, was used to select the optimal *K*, according to Evanno et al. (2005). In order to check the relationships among altitudes and genetic diversity indexes (the observed number of alleles *N*a, the effective number of alleles *N*e, the Nei's gene diversity *H*, the Shannon's information index *I*, and the percentage of polymorphic bands *PPB*), the regression analysis was done by using Origin version 9.1 software (http:// www. OriginLab. com).

Conclusion

The level of genetic diversity of *C. heterophylla* populations in Xingtangsi forest park in Huoshan was high, and the genetic variation mainly existed within populations. The genetic diversity of all eight studied populations indicated a low-high-low pattern of variation along elevation gradients. The genetic diversity of mid-elevation populations were more genetically diverse than both low-elevation and high-elevation populations. The biological characteristics, effective gene flow, and environmental heteroplasmy of *C. heterophylla* influenced the genetic distribution pattern of *C. heterophylla* populations in Xingtangsi forest park.

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References

Abedian M, Talebi M, Golmohammdi HR, Sayed-Tabatabaei BE (2012) Genetic diversity and population structure of mahaleb cherry (*Prunus mahaleb* L.) and sweet cherry (*Prunus avium* L.) using SRAP markers. Biochem Syst Ecol. 40:112-117.

Amar M, Biswas M, Zhang Z, Guo W (2011) Exploitation of SSR, SRAP and CAPS-SNP markers for genetic diversity of *Citrus* germplasm collection. Sci Hort. 128: 220-227.

Aradhya KM, Mueller-Dombois D, Ranker TA (1993) Genetic structure and differentiation in *Metrosideros polymorpha* (Myrtaceae) along altitudinal gradients in Maui, Hawaii. Genet Res. 61: 159-170.

Bellusci F, Pellegrino G, Palermo AM, Argano DG, Musacchio A (2005) Genetic differentiation of the endemic orophyte *Campanula pollinensis* along an altitudinal gradient. Plant Biosyst. 139: 349-356.

Budak H, Shearman RC, Parmaksiz I, Gaussion RE, Riordan TR, Dewiak I (2004) Molecular characterization of buffalograss germplasm using sequence-related amplified polymorphism markers. Theor Appl Genet. 108: 328-334.

Byars SG, Parsons Y, Hoffmann AA (2009) Effect of altitude on the genetic structure of an Alpine grass, Poahiemata. Ann Bot. 103: 885-899.

Cai XY, Feng ZY, Zhang XX, Xu W, Hou BW, Ding XY (2011) Genetic diversity and population structure of an endangered Orchid (*Dendrobium loddigesii* Rolfe) from China revealed by SRAP markers. Sci Horti. 129: 877-881.

Chen G, Wang Y, Zhao C, Korpelainen H, Li C (2008) Genetic diversity of *Hippophae rhamnoides* populations at varying altitudes in the Wolong Natural Reserve of China as revealed by ISSR markers. Silvae Genet. 57 (1): 29-36.

Cheng YH, Miao YM, Bi RC (2012) Study on community floristic characteristics and distribution pattern of *Corylus heterophylla* community in Huoshan of Shanxi Province. J Shanxi Normal University. 26 (1): 71-74.

Dong LG, Wang H (2009) Study on eco-hydrological characteristics of soil of Taishan hazelnut. Shandong Forest Sci Tech. 2: 52-54.

Dong P, Wei YM, Chen GY, Li W, Wang JR, Nevo E, Zheng YL (2010) Sequence-related amplified polymorphism (SRAP) of wild emmer wheat (*Triticum dicoccoides*) in Israel and its ecological association. Biochem Syst Ecol. 38: 1-11.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of cluster of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 14: 2611-2620.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. Genetics. 164: 1567-1587.

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes. 7:574-578.

Feng FJ, Wang FY, Chang SL (2004) Genetic differentiation of *Pinus koraiensis* under different altitude conditions in Changbai Mountains. J Northeast Forestry University. 32 (3): 54-57.

Ferriol M, Pico B, Nuez F (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theor Appl Genet. 107 (2): 271-282.

Gao LX (2012) Studies on quantitative ecology of *Corylus heterophylla* communities in Shanxi. Master Degree's Thesis, Shanxi Normal University.

Gehring JL, Delph LF (1999) Fine-scale genetic structure and clinal variation in *Silene acaulis* despite high gene flow. Heredity. 82: 628-637.

Hahn T, Kettle CJ, Ghazoul J, Frei ER, Matter P, Pluess AR (2012) Patterns of genetic variation across altitude in three plant species of semi-dry grasslands. PLoS One. 7(8): e41608.

Huang CQ, Zhang YF, Liu GD, Bai CJ, Wang WQ (2012) Genetic diversity of *Cynodon radiatus* assessed by sequence-related amplified polymorphism markers. Biochem Syst Ecol. 40: 56-61.

Huang W, Sun P, Zhang WS, Meng FY, Wang YY, Feng CQ (2008) Genetic diversity of *Buplerum Chinese* DC. populations from different altitudes in Dongling Mountain district in Beijing. J Plant Genet Resour. 9(4): 453-457.

Jin ZX, Li JM (2005) Analysis of the genetic diversity of *Hepatacodium micniodes* at different altitude in Tiantai mountain in Zhejiang Province and its relationship with environmental factors. J Zhejiang University. 32(4): 452-458.

Li C, Xu G, Zang R, Korpelainen H, Berninger F (2007) Sex-related differences in leaf morphological and physiological responses in *Hippophae rhamnoides* along an altitudinal gradient. Tree Phys. 27: 399-406.

Li D, Peng SL (2001) Genetic diversity in three *Pinus massoniana* populations in different elevations and its relationship with ecological factors. Acta Ecol Sin. 21 (3): 415-421.

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet. 103: 455-461.

Li J (2010) High yield of enclosure transformation of natural *Corylus heterophylla* forest and its benefit analysis. Prot Forest Sci Tech. 6: 100-101.

Li XQ, Lu YJ, Sun Y (2010 a) Cultivation technique of *Corylus heterophylla*. Jilin Agric. 8: 110.

Li XX, Liu CC, Zhang WD, Shao H, Wu HM, Wang Z, Yang YN, Li JL (2010 b) Studies on cold resistance of Hazel determined and analyzed by Atomic Absorption Spectrometry. Spectrosc Spect. Anal. 6: 228-231.

Lin ZX, Zhang YX, Zhang XL, Guo XP (2009) A high-density integrative linkage map for *Gossypium hirsutum*. Euphytica. 166: 35-45.

Liu LW, Zhao LP, Gong YQ, Wang MX, Chen LM, Yang JL, Wang Y, Yu FM, Wang LZ (2008) DNA fingerprinting and genetic diversity analysis of late-bolting radish cultivars with RAPD, ISSR and SRAP markers. Sci Hort. 116: 240-247.

Liu Z, Zhao A, Kang X, Zhou S, López-Pujol J (2006) Genetic diversity, population structure, and conservation of *Sophora moorcroftiana* (Fabaceae), a shrub endemic to the Tibetan Plateau. Plant Biol. 8: 81-92.

Mcdermott JM, Mcdonald BA (1993) Gene flow in plant pathosystems. Ann Rev Phytop. 31: 353-373.

McMahon SM, Harrison SP, Armbruster WS, Bartlein PJ, Beale CM, Edwards ME, Kattge J, Midgley G, Morin X, Prentice C (2011) Improving assessment and modelling of climate change impacts on global terrestrial biodiversity. Trends Ecol Evol. 26: 249-259.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA. 70: 3321-3323.

Oyama K, Ito M, Yahara T, Ono M (1993) Low genetic differentiation among populations of *Arabis serrata* (Brassicaceae) along an altitudinal gradient. J Plant Res. 106: 143-148.

Peakall R, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel Population genetic software for teaching and research. Mol Ecol Notes. 6(1): 288-295.

Peng S, Feng N, Guo ML, Chen YH, Guo QH (2008) Genetic variation of *Carthamus tinctorius* L. and related species revealed by SRAP analysis. Biochem Syst Ecol. 36: 531-538.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Pritchard JK, Wen W (2004) Documentation for the STRUCTURE software version 2.0. Chicago. http://www.pritch.bsd.uchicago.edu/soft ware/structure2_1. html.

Qiao LX, Liu HY, Guo BT, Weng LM, Dai JX, Duan DL, Wang B (2007) Molecular identification of 16 Porphyra lines using sequence-related amplified polymorphism markers. Aquat Bot. 87: 203-208.

Schneller J, Liebst B (2007) Patterns of variation of a common fern (*Athyrium filix-femina*; Woodsiaceae): population structure along and between altitudinal gradients. Am J Bot. 94(6): 965-971.

Seman K, Jornstad AB, Stedje B (2003) Genetic diversity and differentiation in Ethiopian populations of *Phytolacca dodecandra* as revealed by AFLP and RAPD analyses. Genet Resour Crop Evol. 50: 649-661.

Shi XQ, Song XQ, Song HC, Tang TQ, Fu HW, Wang BL, Yang XB (2005) RAPD analysis on the *Liquidambar formosana* at different altitudes and under different sunlight. Natur Sci J Hainan University. 23(2): 122-126.

Sun ZD, Wang ZN, Tu JX, Zhang JF, Yu FQ, Mcvettyoette BE, Li GY (2007) An ultradense genetic recombination map for *Brassica napus*, consisting of 1355 SRAP markers. Theor Appl Genet. 114: 1305-1317.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24: 1596-1599.

Tao J, Zhang JL, Li TC, Hui RH, Hou DY (2006) Analysis of fatty acids in two kinds of hazelnuts by capillary gas chromatography-mass spectrography. Food Sci Tech. 12: 163-166.

Truong C, Palmé AE, Felber F (2007) Recent invasion of the mountain birch *Betula pubescens* ssp. *tortuosa* above the treeline due to climate change: genetic and ecological study in northern Sweden. J Evolution Biol. 20: 369-380.

Wang GL, Fang HJ (1998) Principle and technology of plant genetic engineering. Beijing: Science Press, China.

Wright S (1931) Evolution in mendelian population. Genetics. 16: 97-159.

Yan N, Wang D, Gao YH, Hao XJ, Wang YL (2010) Genetic diversity of *Acer ginnala* populations at different elevation in Qiliyu based on ISSR markers. Sci Silv Sin. 46(10): 50-56.

Yang M, Han YN, Xu LM, Zhao JR, Liu YL (2012) Comparative analysis of genetic diversity of lotus (*Nelumbo*) using SSR and SRAP markers. Sci Horti. 142: 185-195.

Yeh F, Yang RC, Boyle T, Ye ZH, Mao JX (1997) POPGENE, the user friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Edmonton, Canada.

Young AG, Hill JH, Murray BG (2002) Breeding system, genetic diversity and clonal structure in the subalpine forb *Rutidosis leiolepis* F. Muell. (Asteraceae). Biol Conserv. 106: 71-78.

Zhang X, Orpelainen HK, Li C (2006) Microsatellite variation of *Quercus aquifolioides* populations at varying altitudes in the Wolong Natural Reserve of China. Silva Fenn. 40: 407-415.

Zhao LF, Li S, PanY (2001) *Psathyrostachys* genetic differentiation of natural populations along an altitudinal gradient. Acta Bot Boreali-Occidentalia Sin. 21 (3): 391-400.