JSE Journal of Systematics and Evolution

Journal of Systematics and Evolution 52 (2): 175–185 (2014) doi: 10.1111/jse.12065

Research Article

Phylogeography of the widespread plant *Ailanthus altissima* (Simaroubaceae) in China indicated by three chloroplast DNA regions

[1,2]Yi-Ying LIAO [1]You-Hao GUO [3]Jin-Ming CHEN* [3]Qing-Feng WANG*

[1](*Laboratory of Plant Systematics and Evolutionary Biology*, *College of Life Sciences, Wuhan University*, Wuhan 430072, China)
[2](*Fairy Lake Botanical Garden, Shenzhen*, *Chinese Academy of Sciences*, Shenzhen 518004, China)
[3](*Key Laboratory of Aquatic Botany and Watershed Ecology*, *Wuhan Botanical Garden, Chinese Academy of Sciences*, Wuhan 430074, China)

Abstract *Ailanthus altissima* (Mill.) Swingle, a temperate tree species, has a wide distribution in China. To infer its refugia and patterns of migration during past climatic changes in China, genetic variations among different populations were studied. Gene sequences of three chloroplast DNA spacer regions, *psbA-trnH*, *trnL-trnF*, and *trnD-trnT*, were obtained from 440 individuals of 44 populations. The distribution of haplotype and the relationships among them were investigated by haplotype network. In addition, the genetic diversity of the sampled regions was inferred, and the biogeographic history was also reconstructed. Twelve haplotypes were identified, among which, five were unique. The phylogenetic analysis and geographical distribution of haplotypes indicate that multiple glacial refugia existed in mainland China during the Quaternary oscillations. Due to the combined effects of contiguous range expansion and allopatric fragmentation, significant genetic structure was not found in this study. Based on biogeographic and demographic analysis, three main dispersal routes were identified for the major haplotypes, whereas others were more likely localized demographic expansion.

Key words *Ailanthus altissima*, cpDNA, glacial refugia, migration, phylogeography.

Phylogeography, which aims to understand the historical colonization process within and among species in the light of contemporary geographical distributions and gene genealogy, has been regarded as one major focus of evolutionary biology in recent years (Avise, 2000). Over the past decade, numerous studies have been carried out to trace the spatial and genealogical distribution of genetic variation at intraspecific level or among closely related species (e.g., Avise, 2000, 2008; Hickerson et al., 2010; Qiu et al., 2011). They have found that climate oscillations have a vital influence on species' current distribution. For example, tree lines shifted southwards and to lower elevations during periods of cold climate, and retreated during warmer intervals (Birks, 2008; Marr et al., 2013). Although East Asia, especially mainland China, was not covered by ice sheets during the last glacial period, climate oscillations during the late Quaternary strongly affected the vegetation (Marr et al., 2013).

Regional expansions and intraspecific divergences were common for many species during the Quaternary oscillations (Qiu et al., 2011; Liu et al., 2012). In mainland China, different regions with the large latitudinal span (22°N–42°N) and the complex terrain patterns were impacted at different levels by the cold ice age. The predominant biotic response of temperate plant species to Quaternary environmental change includes range fragmentation, vicariance, and population isolation (Comes et al., 2008; Yesson et al., 2009). Recent studies on the phylogeography of some plant species, such as *Myricaria* Desv. (Liu et al., 2009), *Cyananthus delavayi* Franch. (Li et al., 2012), *Lagochilus* Bunge ex Benth. (Meng & Zhang, 2013), and *Bupleurum longiradiatum* Turcz. (Zhao et al., 2013), suggested that the footprints of historical events such as contraction and expansion of the distribution were caused by climate oscillations during the Quaternary Era (Shimono et al., 2013).

The phylogeography of a diverse array of temperate plant species in mainland China and its adjacent areas has been investigated, for example, *Ligularia vellerea* Hand.-Mazz. in the Hengduan Mountains (Yang et al., 2012), *Clematis sibirica* Mill. in the Tianshan and Altai Mountains (Zhang &

Received: 5 April 2013 Accepted: 18 July 2013
* Authors for correspondence. JM CHEN. E-mail: jmchen@wbgcas.cn. Tel.: 86-27-87617212. Fax: 86-27-87510526. QF WANG. E-mail: qfwang@wbgcas.cn. Tel./Fax: 86-27-87510526.

Zhang, 2012), *Sophora davidii* Kom. ex Pavol. in southwest China (Fan et al., 2013). The studies showed that multiple glacial refugia seem to be a general rule for most species, and the major glacial refugia in China were found to be located at a few large mountains (Qiu et al., 2011; Liu et al., 2012). Landscape features in mainland China also played an important role in shaping the genetic structure of plant species, for example, many species have strong phylogeographic breaks even over relatively short geographic distances when associated with complex topography of the region (Gao et al., 2007; Zhang et al., 2011).

Ailanthus altissima (Mill.) Swingle is an important broad-leaved tree in East Asia and distributes widely in the Northern Hemisphere at a minimum age of the early Eocene according to fossil records (Corbett & Manchester, 2004). Its wide distribution in China extends from the northern edge of Liaoning Province to the southern end of Guangxi Province. *Ailanthus altissima* is a pioneer species, which can endure a high level of natural and human disturbance. For example, it can survive in extreme environments, such as temperatures as low as –33 °C, and can grow in various habitats from rocky mountains, sterile soils, to fertile earth (Kowarik & Säumel, 2007). It propagates by producing wind-dispersed seeds and by vegetative reproduction via sprouts (Kowarik & Säumel, 2007). *Ailanthus altissima* can produce as many as 300 000 seeds in one season (Aldrich et al., 2010), so they are capable of colonizing open spaces quickly, such as roadsides, fallow fields, and abandoned land. Owing to its pioneer characteristics, *A. altissima* is widely planted in arid and semiarid regions in North China. Moreover, this plant has become an invasive species in Europe and North American due to its fast growth and tolerance (Burch & Zedaker, 2003). Therefore, the knowledge of genetic variation of *A. altissima* in native populations is necessary for future use and management of this species. Although the genetic structure of *A. altissima* in the eastern USA (Aldrich et al., 2010) and the genetic diversity in Japan (Kurokochi et al., 2013) have been investigated, little is known about the phylogeographic patterns and population genetics of this species in China.

In this study, we used chloroplast DNA (cpDNA) to investigate the genetic variation of *A. altissima*, in which the geographical variation of cpDNA is expected to reflect patterns of historical seed flow and colonization. Our sampling covered most of the range of this species in China. Due to the wide distribution, we investigated populations from various climate conditions, from cool-temperate deciduous forest in North China, to warm-temperate evergreen forest in subtropical areas. We mainly focused on the genetic structure and evolutionary history of the pioneer species in China. Three major issues will be addressed: (i) the genetic diversity of this species and the spatial pattern of cpDNA haplotypes in China; (ii) the locations of main glacial refugia in China during glaciation; and (iii) the migration history of *A. altissima* contributing to its current distribution.

1 Material and methods

1.1 Sampling and DNA extraction

We sampled plant material of *Ailanthus altissima* from 44 populations in 14 provinces, covering most of its distribution areas in China (Table 1; Fig. 1). In consideration of human propagation, we selected older trees growing in montane forests whenever possible. In each population, samples of 20 individuals were collected at an interval of 50 m or more to avoid collecting genetically identical individuals. Leaves were dried with silica gel for DNA extraction. In this study, we randomly chose 10 samples from one population for analysis. Total genomic DNA was extracted using the modified CTAB buffer method (Doyle & Doyle, 1987). The isolated DNAs were dissolved in TE buffer and stored at −20 °C before use.

1.2 Sequencing of non-coding regions of cpDNA

Gene sequences of three non-coding plastid regions (*psbA-trnH*, *trnL-trnF*, and *trnD-trnT*) were obtained in this study. The amplification and sequencing primers of the three regions were as follows: *psbA-trnH*, 5′-GTTATGCATGAACGTAATGCTC-3′ and 5′-CGCGCATGGTGGATTCACAATC-3′ (Sang et al., 1997); *trnL-trnF*, 5′-GGTTCAAGTCCCTCTATCCC-3′ and 5′-CGCGCATGGTGGATTCACAATC-3′ (Taberlet et al., 1991); and *trnD-trnT*, 5′-ACCAATTGAACTACAATCCC-3′ and 5′-CTACCACTGAGTTAAAAGGG-3′ (Demesure et al., 1995). Polymerase chain reactions (PCR) were carried out in a total volume of 50 μL consisting of 35 μL de-ionized water, 0.25 mmol/L each dNTP, 5 μL of 10× Taq buffer (10 mmol/L Tris–HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, and 50 mmol/L KCl), 1 mmol/L each primer, 2 U *Taq* polymerase (TransGen Biotech, Beijing, China), and 60 ng DNA template. The program was set as an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min annealing at 53 °C, and 80 s extension at 72 °C, with a final extension of 7 min at 72 °C. All PCR products were purified from agarose gel with the Tian quick Midi Purification Kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions. The purified

Table 1 Location of *Ailanthus altissima* sampled in this study

Populations	Localities (all in China)	Coordinates (N, E)	Regions	Haplotype
1. LH	Lanhansuo, Gansu	103°51′, 36°03′	A	1
2. LY	Yantan park, Gansu	103°51′, 36°31′	A	1
3. TJ	Jiugongshan, Hubei	114°51′, 29°24′	B	2
4. YT	Yichangtucheng, Hubei	111°06′, 30°39′	C	3
5. DY	Dangyang, Hubei	111°47′, 30°49′	C	3
6. ZJ	Zhijiang, Hubei	111°35′, 30°31′	C	2
7. HZS	Hanzhong, Shaanxi	107°00′, 33°04′	A	1
8. DLL	Dalaoling, Hubei	110°55′, 31°03′	C	4
9. CY	Changyang, Hubei	110°52, 30°39′	C	5
10. MLZ	Mulinzi, Hubei	110°12′, 30°02′	C	5
11. QD	Qingdao, Shandong	120°31′, 36°10′	A	6
12. TS	Tai′an, Shandong	117°07′, 36°15′	A	7
13. CX	Cixian, Hebei	114°21′, 36°21′	A	1
14. HD	Handan, Hebei	114°28′, 36°36′	A	1
15. LX	Lanxian, Shanxi	111°40′, 38°16′	A	1
16. SD	Suide, Shaanxi	110°15′, 37°29′	A	3
17. JB	Jingbian, Shaanxi	108°39′, 37°16′	A	1
18. YC	Yuncheng, Shanxi	111°01′, 34°58′	A	1
19. JX	Jiexiu, Shanxi	111°57′, 36°53′	A	1
20. PY	Pingyao, Shanxi	112°60′, 37°12′	A	1
21. PX	Wugongshan, Jiangxi	114°08′, 27°28′	B	2
22. NP	Nanping, Fujian	118°10′, 26°38′	B	7
23. WYS	Wuyishan, Fujian	117°40′, 27°44′	B	2
24. SC	Shucheng, Anhui	117°11′, 31°31′	B	7
25. CZ	Chuzhou, Anhui	118°17′, 32°18′	B	7
26. NJ	Zijinshan, Jiangsu	118°50′, 32°03′	B	8
27. XXS	Xixiashan, Jiangsu	118°57′, 32°09′	B	7
28. ZZY	Suzhou, Jiangsu	120°37′, 31°19′	B	8
29. SFS	Shangfangshan, Jiangsu	120°35′, 31°15′	B	9
30. LA	Linan, Zhejiang	119°13′, 30°12′	B	8
31. HZ	Hangzhou, Zhejiang	120°06′, 30°14′	B	8
32. ZS	Zhoushan, Zhejiang	122°23′, 29°52′	B	10
33. JD	Jande, Zhejiang	119°07′, 29°26′	B	11
34. JDZ	Jingdezhen, Jiangxi	117°17′, 29°19′	B	8
35. XS	Xiangshan, Beijing	116°18′, 39°58′	A	7
36. TBY	Nanyang, Henan	113°17′, 32°24′	A	3
37. LS	Lushi, Henan	111°03′, 34°03′	A	1
38. XX	Xixia, Henan	111°45′, 33°38′	A	1
39. LB	Lingbao, Henan	110°40′, 34°31′	A	1
40. DZP	Longtanjiang, Guangxi	110°51′, 25°08′	C	12
41. GZ	Gaozhai, Guangxi	110°27′, 25°47′	C	12
42. HP	Huaping, Guangxi	109°55′, 25°37′	C	12
43. MEK	Maerkang, Sichuan	102°13′, 31°54′	D	1
44. XC	Xiangchen, Sichuan	99°52′, 29°01′	D	1

Four regions were defined based on the regionalization of Chinese flora (Wu, 1979): A, North China Region; B, East China Region; C, Central China Region; and D, Hengduan Mountains Region.

PCR products were sequenced using the ABI Prism BigDye terminator cycle sequencing ready reaction kit in an ABI 3730 automated sequencer (both Applied Biosystems, Foster City, CA, USA).

1.3 Data analysis

The sequences of the three cpDNA non-coding regions were aligned using CLUSTALX 2.0 (Larkin et al., 2007) then adjusted manually with BioEdit version 7.0.0 (Hall, 1999). They were then concatenated into one matrix. The cpDNA haplotypes were determined by nucleotide substitutions and indels of the aligned sequences with DnaSP 4.0 (Rozas et al., 2003). Haplotype diversity (h), nucleotide diversity per population (π) (Nei & Tajima, 1983), Tajima's D (Tajima, 1989), Fu and Li's D^* (Fu & Li, 1993), and the mismatch distribution (Rogers & Harpending, 1992) with constant population size as model for expected values were also estimated by DnaSP 4.0. To detect genetic variation between populations and molecular variation of haplotype distribution (F_{ST}) (Weir & Cockerham, 1984), the molecular variation analysis was carried out by analysis of molecular variance (Excoffier et al., 1992) implemented in Arlequin 3.0 (Excoffier et al., 2005). The correlation of the genetic distance and geographical distance between populations was evaluated by isolation by distance (IBD3.22; Bohonak, 2002), in which the geographical distances between populations were calculated with the aid of PASSaGE version 2.0 (Rosenberg & Anderson, 2011).

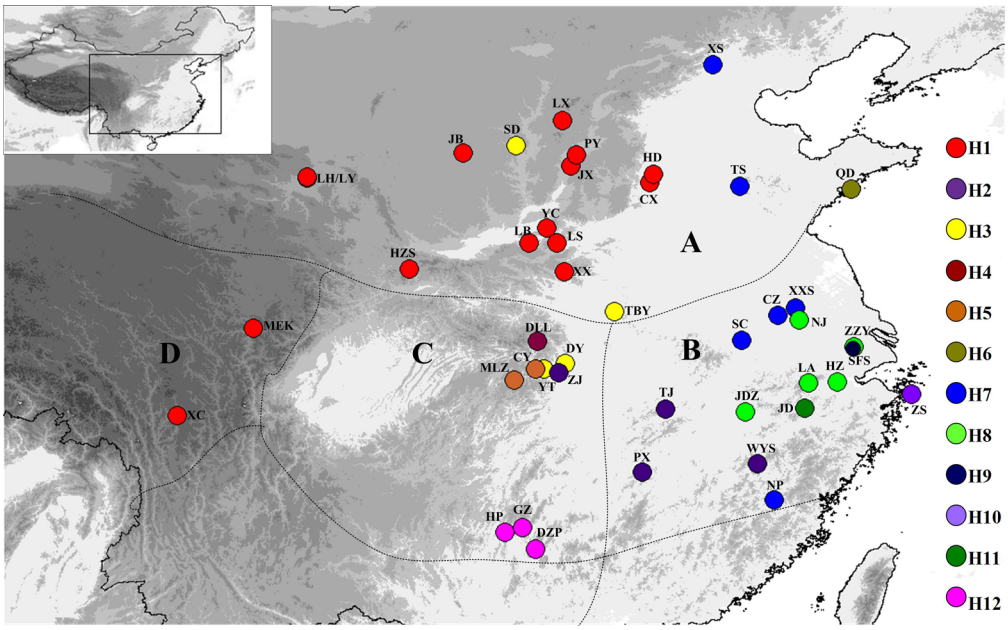

Fig. 1. Geographical distribution map for *Ailanthus altissima* haplotypes (H1–H12) based on cpDNA sequence variation from 44 natural populations sampled in China. Distributions of *A. altissima* in China were partitioned into four regions according to Wu (1979): A, North China Region; B, East China Region; C, Central China Region; D, Hengduan Mountains Region. The distribution map was modified from data available from free spatial data available from DIVA-GIS (http://www.diva-gis.org/gdata). Population codes correspond to those in Table 1.

Phylogenetic relationships were inferred using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. Both MP and ML were carried out by PAUP* version 4.0 (Swofford, 2003). The MP analyses involved a heuristic search strategy with 1000 replicates of random addition of sequences, in combination with tree bisection–reconnection branch-swapping on all the resulting trees with the MulTrees options in effect. All characters were set to "unordered" and had equal weight. Gaps were treated as multistate characters. Branch support was assessed by bootstrap analysis with 1000 replicates. For ML and Bayesian analyses, F81 was determined as the best-fitting model with Modeltest 3.7 (Posada, 2005) and MrModeltest (Nylander, 2004). The ML analysis was based on heuristic searches with the tree bisection–reconnection branch-swapping algorithm. The robustness of the trees was assessed by bootstrapping 1000 random replicates. Bayesian analysis was carried out using MrBayes (Huelsenbeck & Ronquist, 2001). Every 100th tree was sampled over six Mio generations with the first 25% of trees were treated as burn-in.

Based on the matrix of pairwise differences between cpDNA haplotypes, a statistical parsimony haplotype network was obtained with the aid of TCS 1.06 (Clement et al., 2000) by using the 95% connection probability limit and treating gaps as single evolutionary events. Relationships among chloroplast haplotypes were also investigated. In addition, we carried out a nested analysis using AeNCA (Panchal, 2007), to get the nested cladogram of genetic clades.

To reconstruct the biogeographic history of *A. altissima* in China, dispersal–vicariance analysis was carried out by DIVA version 1.1 (Ronquist, 1996). Each haplotype was set to one or more areas based on its current distribution and each taxon was scored as presence (1) or absence (0). Distribution of *A. altissima* in China was partitioned into four areas according to Wu (1979): A, North China Region; B, East China Region; C, Central China Region; and D, Hengduan Mountains Region (Fig. 1). Analyses were implemented in the ML tree using the cpDNA haplotype sequences. Due to the strict bifurcation tree requirement in DIVA analysis, we treated the ML tree as a topological structure completely solved (the unsolved end branches were combined). The maximum number of areas at each node in DIVA was set to two.

2 Results

The size of aligned *psbA-trnH*, *trnL-trnF*, and *trnD-trnT* regions are 491, 581, and 511 bp, respectively. The total length of the concatenated alignments is 1583 bp. Nine nucleotide substitutions and 21 indels were identified. Based on the variations, 12 haplotypes were identified (Fig. 1; Table 1). The sequences of these

haplotypes were deposited into GenBank (Accession Nos. KC816424–KC816459).

At the species level, the haplotype diversity and nucleotide diversity are moderate, with the h_T and π_T being 0.729 and 0.00140, respectively, a little lower than those of other temperate plants, such as *Ostryopsis davidiana* Decne. (Tian et al., 2009), *Dysosma versipellis* (Hance) M. Cheng ex T. S. Ying (Qiu et al., 2009a), and *Eurycorymbus cavaleriei* (H. Lév.) Rehder & Hand.-Mazz. (Wang et al., 2009), but much higher than that of *Picea crassifolia* Komarov (Meng et al., 2007). Nevertheless, no cpDNA polymorphism was detected among individuals from the same population. Tajima's *D* and Fu and Li's tests indicated non-significant deviation from neutrality for all populations. Furthermore, the polymodal distribution curve was uncovered by mismatch distribution analysis. Therefore, no evidence was detected for recent demographic expansions of *Ailanthus altissima*.

In total, 12 haplotypes were identified for 44 populations (Fig. 1), 7 of which are common. The most widespread haplotypes were H1 (15 populations), H7 (6 populations), and H8 (5 populations). In addition, H2, H3, H5, and H12 occurred in more than one population (Fig. 1; Table 1). Haplotypes H1, H2, H3, and H7 were found distributed in two regions (Fig. 1). In contrast, H4, H6, H9, H10, and H11 were found in only one population, and thus were considered as unique haplotypes. Therefore, unique haplotypes accounted for 41.7% of all haplotypes, almost comparable to the number of common haplotypes. Hierarchical analysis of molecular variance indicated a high degree of differentiation among populations ($F_{ST} = 1$), and a lower level of differentiation (28.78%) among regions. If compared within regions, there was a great amount of variation (71.22%) among populations, and none within populations (Table 2). The Mantel test suggested that genetic distance was uncorrelated with geographic distance ($r = 0.1689$, $P = 0.9880$), thus no significant genetic structure was detected.

Phylogenetic analyses resulted in one identical topology using MP, ML, and Bayesian inference, and three clades were identified (Fig. 2). The bootstrap value ranged from 57% to 88%, and posterior probability values were also relatively high. Three haplotypes (H8, H10, and H11) from East China formed a strongly supported clade; H2 and H7 formed another subclade (Fig. 2). The unrooted TCS network of cpDNA haplotypes (Fig. 3) was consistent with the topology revealed by phylogenetic analyses. In the TCS network of 12 haplotypes, H1, H2, H5, H6, and H8 were the interior haplotypes, which were regarded as

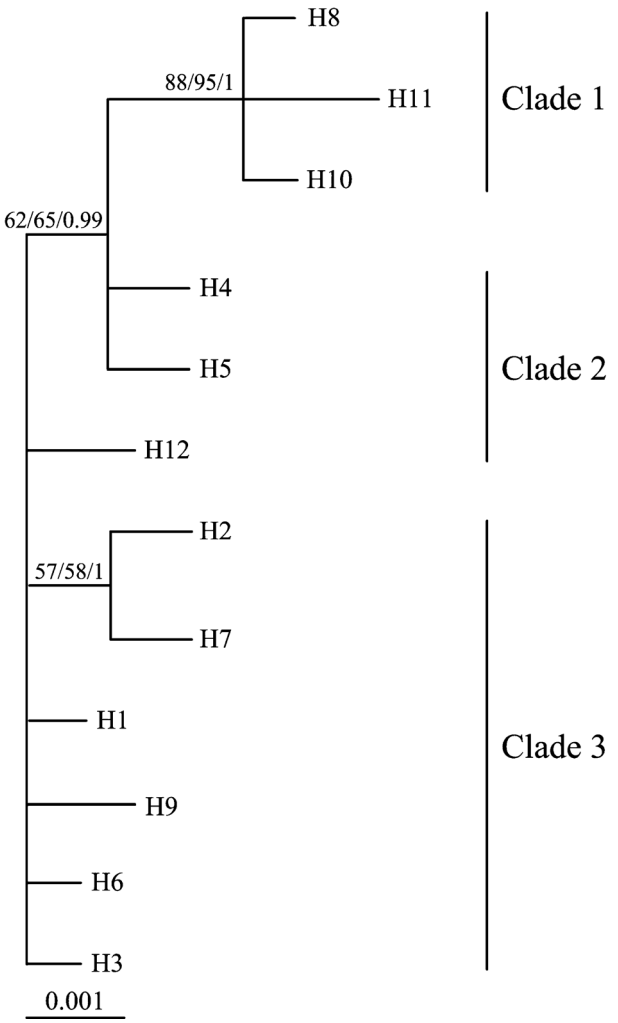

Fig. 2. Chronogram indicating the evolutionary relationships among chloroplast haplotypes found in *Ailanthus altissima* from mainland China. Numbers above the branches indicate bootstrap and posterior probability values for maximum, parsimony maximum likelihood, and Bayesian inference analyses, respectively.

Table 2 Results of analysis of molecular variance of *Ailanthus altissima* populations, partitioned by regions

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation
Among groups	3	29.691	0.13369	28.78
Among populations within groups	40	74.400	0.33083	71.22
Within populations	220	0.000	0.00000	0.00
Total	263	109.091	0.46452	

$F_{ST} = 1.0000$.

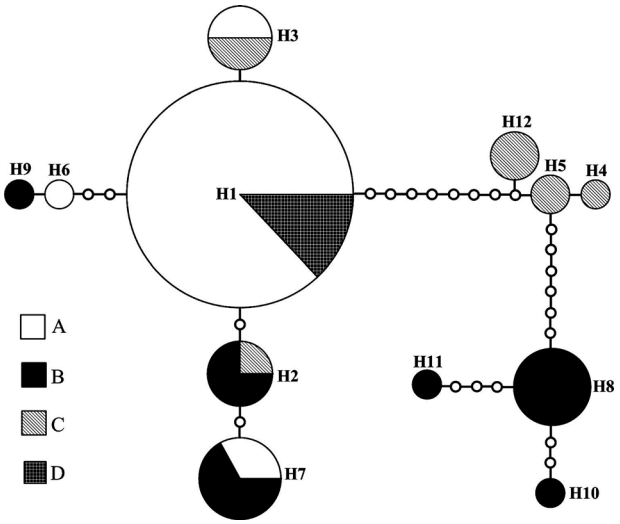

Fig. 3. Haplotype network of chloroplast DNA haplotypes H1–H12 found in *Ailanthus altissima* from mainland China, constructed by TCS 1.06. Circle size and circle sector size are proportional to the number of individuals possessing each haplotype. Each line represents single base mutations, open circle represents an inferred intermediate haplotype. A, North China Region; B, East China Region; C, Central China Region; D, Hengduan Mountains Region.

ancestral to tip haplotypes. The minimum-spanning network analysis indicated 17 one-step clades, 8 two-step clades, and 3 three-step clades in the haplotype network (Fig. 4). Three groups were formed in this network with their distribution ranges overlapped geographically. For the nested clade analysis (NCA),

Table 3 Inference chain of population structure and history

Clade	Chain of inference	Inference result
Clade 2-1	1-19-20-2-11-12 NO	Contiguous range expansion
Clade 3-1	1-19-20-2-11-12 NO	Contiguous range expansion
Clade 3-3	1-19 NO	Allopatric fragmentation
Total cladogram	1-2-11-12 NO	Contiguous range expansion

four clades (2-1, 3-1, 3-3, the total) were identified as having significant phylogeographical structure, making the inference of historical and ongoing processes possible (Table 3). The NCA suggested that contiguous range expansion contributed to the current haplotype distribution of different clades, that is, (i) clade 2-1 (H2, H7), most of the populations were from East China, except for ZJ from the Dabashan mountains (Mts.); (ii) clade 3-1 (H1, H2, H3, H6, H7, H9), populations spread across the mainland China; and (iii) the total clade, all the populations sampled in this study. The NCA also indicated that allopatric fragmentation was responsible for the population differentiation in clade 3-3 (H4, H5, H12), in which populations of H4 and H5 were from the Dabashan Mts., and the H12 population was from the Nanling Mts. In summary, our results suggested that discontinuous range expansion was responsible for the present distribution at all populations, and that allopatric fragmentation resulted in the division of local populations.

The reconstruction of the ancestral geographical distribution areas of genetic lineages based on the dispersal–vicariance analyses is shown in Fig. 5. The

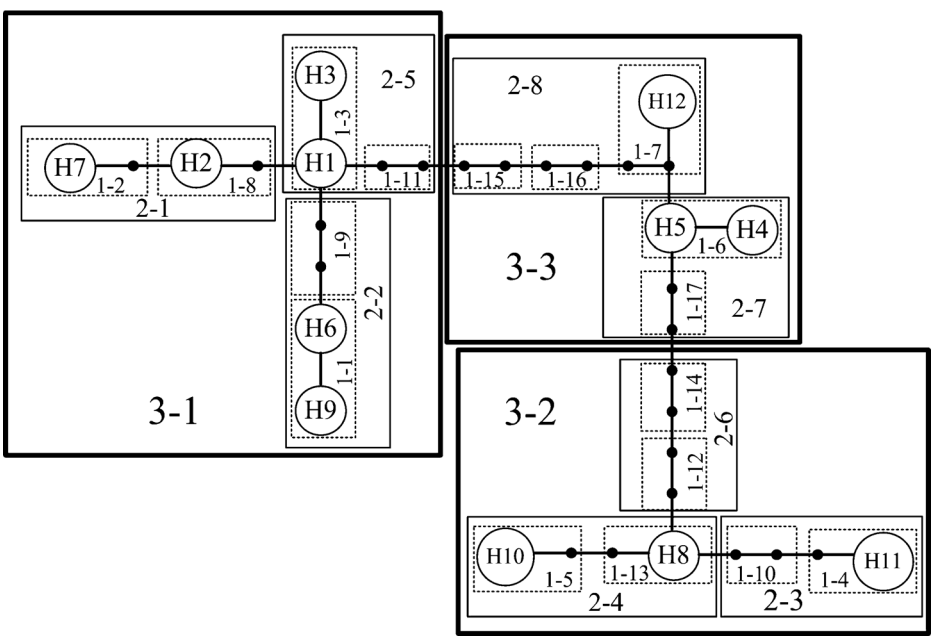

Fig. 4. Nested cladogram of 12 haplotypes based on chloroplast DNA sequences of *Ailanthus altissima* found in mainland China. Cycles with numbers (H1–H12) represent haplotypes. Bold circles represent putative haplotypes. Each branch represents one mutation.

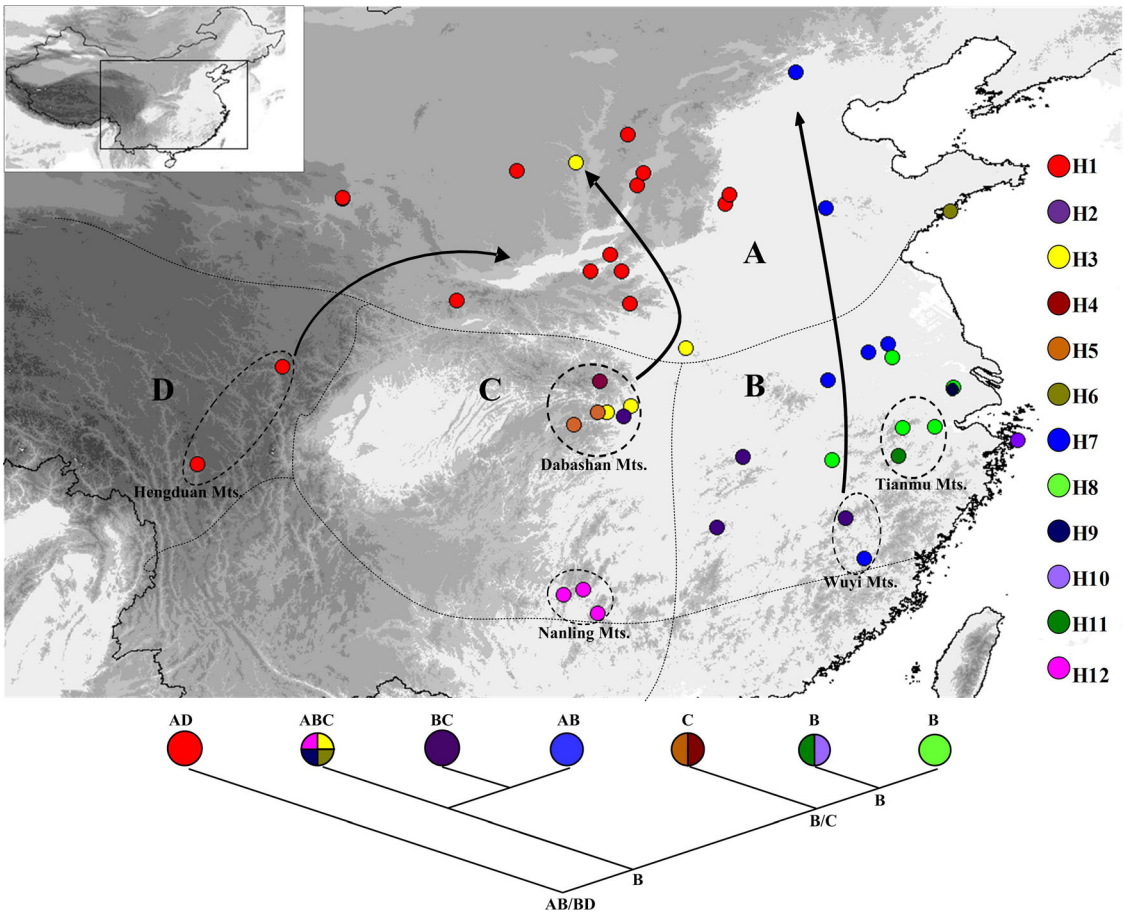

Fig. 5. Distribution of extant haplotypes, location of putative refugia (dashed circles), and putative expansion routes (arrows) of *Ailanthus altissima* in mainland China, and the result of *A. altissima* dispersal–vicariance analyses. Letters below nodes note DIVA reconstructions as follows: A, North China Region; B, East China Region; C, Central China Region; D, Hengduan Mountains Region. Those letters refer to DIVA reconstructions under the "maximum of two ancestral areas" constraint, and letter combinations divided by a "/" reflect alternative DIVA reconstructions. The distribution map was modified from data available from free spatial data program DIVA-GIS (http://www.diva-gis.org/gdata).

results suggested that the common ancestor was probably distributed in North China (A), East China (B), and the Hengduan Mts. (D). East China was probably the cradle of origin and diversity for *A. altissima*, as the majority of haplotypes, especially the unique haplotypes, were found there.

3 Discussion

3.1 Genetic diversity and population structure

Ailanthus altissima is widely distributed in China. In contrast to the broad geographical range, relatively low levels of nucleotide diversity ($h_T = 0.729$, $\pi_T = 0.0014$) were detected, which is comparable to some gymnosperms in mainland China, such as *Picea crassifolia* (Meng et al., 2007), and *Pinus* L. (Tian et al., 2008). Kurokochi et al. (2013) also detected low chloroplast diversity in populations of *A. altissima* in Japan. However, the genetic variation within populations was extremely low, indicated by the significant population subdivision ($F_{ST} = 1.0$). The results suggested little ongoing seed flow and high susceptibility to random genetic drift in isolated populations (Li et al., 2008). The phylogeographic structure of cpDNA variation in *A. altissima* was similar to a temperate plant, *Ostryopsis davidiana*, distributed in northern China, that is, only a single haplotype of *O. davidiana* was fixed in almost all populations in northern China (Tian et al., 2009). They deduced that *O. davidiana* might have experienced extensive bottleneck or founder effects during glacial population contraction and following postglacial expansions. However, our findings here suggested that allopatric fragmentation and discontinuous range expansion might have promoted intraspecific divergence and differentiation in *A. altissima*.

Due to the widespread and overlapping distribution of some common haplotypes (Fig. 3), genetic

structure was weak in *A. altissima*. We think that frequent localization of genetic variation in *A. altissima* was caused by genetic drift and presumably a long history of dispersal over great distances in mainland China. Many other temperate plant species in mainland China have their common haplotypes with little overlap, such as *Pedicularis longiflora* Rudolph (Yang et al., 2008), *Dysosma versipellis* (Qiu et al., 2009a), *Kirengeshoma* Yatabe (Qiu et al., 2009b), and *Platycarya strobilacea* Sieb. & Zucc. (Chen et al., 2012). Thus, the geographical patterns found in these species revealed locations of glacial refugia and post-Ice Age migration routes. Even though the genetic structure was not significant in *A. altissima*, the geographical structure of haplotypes was obvious (Table 3). The extant distributions of *A. altissima* overlapped with the accepted refugia of species mentioned above. Thus, we were able to trace the glacial refugia and migration patterns of *A. altissima*, according to its present geographical structure of haplotypes and the location of well-accepted refugia.

3.2 Multiple refugia of *A. altissima*

Posada & Crandall (2001) proposed that older alleles could occupy interior nodes of a haplotype network based on coalescent theory. Among the 12 haplotypes, H1, H2, H5, H6, and H8 are the interior haplotypes that could be considered as ancestral (Fig. 4). These common haplotypes show a clear-cut geographical distribution, all being confined to four defined geographical regions, whereas the unique haplotypes are distributed in almost all regions. Moreover, the result of NCA indicated several diverging clades of related haplotypes that correspond fairly well with geography. The above results suggested that *A. altissima* occupied multiple refugia during the last glacial epoch. Due to the extremely low genetic diversity within populations, the precise locations of refugia are difficult to ascertain. Therefore, we used the montane area rather than a specific location of a population as the putative glacial refuge.

It is generally considered that the number of haplotypes decreases with increased distance from refugial sources, unless the area is a contact zone with immigration of different haplotypes from different refugia (Hewitt, 2000; Provan & Bennett, 2008). Species were more likely to have persisted in several glacial refugia, when its extant distributions including multiple refugia are relatively easily dispersed (Shafer et al., 2010; Marr et al., 2013). The genetic evidence of *A. altissima* supports this view. In central and eastern China, many mountains are considered glacial refugia, such as the Dabashan, Tianmushan, Nanling, Hengduan, and Wuyi mountains (Qiu et al., 2011). Marr et al. (2013) suggested that the number of haplotypes in refugium is higher than that in adjacent areas. In this study, the result verified that viewpoint, because the number of common and unique haplotypes in Central and East China, especially in the mountainous areas accepted as refugium, is much higher.

We propose that there may exist multiple refugia in the distribution of *A. altissima*, such as the Tianmushan, Dabashan, Hengduan, Wuyi, and Nanling mountains. The Tianmushan Mts. comprise one major refugium for *A. altissima*, as this region harbors the ancestral haplotype H8 and the unique haplotype H11, according to NCA. The Tianmushan Mts. has high species richness and harbors tertiary species as well as endemic species, and it was supposed to be a glacial refugium of many species such as *Ginkgo biloba* L. (Gong et al., 2008). The Dabashan Mts. was considered to be the second glacial refugium, because one-third of the identified haplotypes, including two ancestral haplotypes (H2, H5), one common haplotype (H3), and one unique haplotype (H4), were detected here. Moreover, the genetic diversity of *A. altissima* in this montane area was higher than that in other regions. The Dabashan Mts. is characterized by its diversity of habitats, high species richness, as well as numbers of endemic species; it was also proposed to be the refugium for *Eurycorymbus cavaleriei* (Wang et al., 2009). Haplotype H12 is closely related to H5 from the Dabashan Mts., but is confined to the Nanling Mts., which is warm and humid, and was affected very little by the Quaternary glaciation. Therefore, the Nanling Mts. could be considered as one possible glacial refugium of *A. altissima*. Likewise, *E. cavaleriei* was proposed to survive here during glaciation (Wang et al., 2009). Harboring abundant endemic and relict species, the Hengduan Mts. was accepted as the refugium of numerous plants such as *Taxus wallichiana* Zucc. (Gao et al., 2007), *Primula secundiflora* Franch. (Wang et al., 2008), and *Tsuga dumosa* Eichl. (Cun & Wang, 2010). We suggest that the Hengduan Mts. might be another refugium of *A. altissima*, because of the existence of an ancestral haplotype H1 in this region. In addition, the distribution of common haplotype H7 ranged from Fujian province (NP) to Beijing (XS) (Fig. 1). Because the population NP was from the Wuyi Mts., which was previously recognized as a refugium (Qiu et al., 2011), the Wuyi Mts. could be a possible refugium of *A. altissima*.

3.3 Migration routes of *A. altissima*

Twelve cpDNA haplotypes were identified in total for *A. altissima* in mainland China; four of them (H1, H2, H3, and H7) distribute broadly and are not endemic to

particular regions or mountains. As inferred by the NCA (Fig. 4; Table 3), contiguous range expansion was the major historical process responsible for the present observed geographical structure of cpDNA haplotypes in *A. altissima*. However, the neutrality tests rejected the recent range expansion as an important process influencing extant cpDNA haplotype structure of *A. altissima*. As *A. altissima* is an important species composing warm-temperate deciduous forests, the above conflicting results could be considered that *A. altissima* experienced continuous expansion over a long evolutionary history rather than sudden postglacial expansion.

Although subtropical mainland China was not covered by ice sheets during the last glacial period (Ono, 1984), climate oscillations throughout the Quaternary glacial had a dramatic impact on species' ranges in these regions (Millien-Parra & Jaeger, 1999; Harrison et al., 2001). Combining the results of DIVA analysis (Fig. 5) with the haplotype network of cpDNA haplotypes (Fig. 3), and the extant distribution of haplotypes (Fig. 1), we propose that North China, East China, and the Hengduan Mts. region were centers of original distribution for *A. altissima*. Some haplotypes (H1, H3, and H7) distribute widely (Fig. 5), and not only occurred in possible refugia (Hengduan Mts., Dabashan Mts., and Wuyi Mts.), but also dispersed to North China. Thus, three main demography expansion routes (Fig. 5) were proposed by reconstructing the past vegetation based on the three common haplotypes (H7, H3, and H1), respectively: (i) the eastern route, from the Wuyi Mts. to the Xiangshan Mts.; (ii) the middle route, from the Dabashan Mts. to the Taihang Mts.; and (iii) the western route, from the Hengduan Mts. to the vast North China region. Another common haplotype, H2, which probably originated in East China based on the DIVA analysis (Fig. 5), is continuously distributed between the Dabashan and Wuyi mountains. One explanation for its distribution pattern is that H2 originated in a southern refugium (Wuyi Mts.) and dispersed to a northern refugium (Dabashan Mts.), and the Quaternary glacial had no dramatic impact on its ranges, so there was no large number of colonizations from the refugium. The vast continental hilly areas in Central and East China harbor complex terrains and diversified habitats, which might lead to the localization of *A. altissima* in different mountains and regions. Thus, allopatric fragmentation was probably responsible for the frequent occurrence of unique haplotypes of *A. altissima*.

3.4 Conclusion and perspective

Ailanthus altissima is well-known as an invasive species and is distributed all over the world. Moderate genetic diversity with little genetic structure was identified in its native range in China. Common and unique haplotypes were widely distributed in mainland China. The extant distribution of *A. altissima* and the haplotype relationships suggested that there were multiple refugia during the Quaternary glacial. Both biogeographic and demographic analyses indicated that three dispersal routes existed over the evolutionary history for some common haplotypes of *A. altissima*, whereas the others were characterized by localized demographic expansion. However, the phylogeographic history of Chinese *A. altissima* is not resolved completely because natural populations collected in this research were limited compared to its broad geographical range. Moreover, we could not distinguish the sources of some populations between human propagation and natural dispersal/migration. Further study is needed with more sampling and more molecular methods, such as nuclear DNA analysis, to confirm the evolutionary history of each haplotype, and provide background information for genetic research of this species around the world.

Acknowledgements We are grateful to Dr. Cai DAN and Dr. Ning ZHANG for their help in manuscript modification. We also thank Jian-Quan LIU for valuable comments on earlier versions of the manuscript, and editors for helping with language revision.

References

Aldrich PR, Briguglio JS, Kapadia SN, Morker MU, Rawal A, Kalra P, Greer GK. 2010. Genetic structure of the invasive tree *Ailanthus altissima* in eastern United States cities. Journal of Botany. doi: 10.1155/2010/795735.

Avise JC. 2000. Phylogeoraphy: The history and formation of species Cambridge: Harvard University Press.

Avise JC. 2008. Phylogeography: Retrospect and prospect. Journal of Biogeography 36: 3–15.

Birks HH. 2008. The late-quaternary history of arctic and alpine plants. Plant Ecology and Diversity 1: 135–146.

Bohonak AJ. 2002. IBD (Isolation by Distance): A program for analyses of isolation by distance. Journal of Heredity 93: 153–154.

Burch PL, Zedaker SM. 2003. Removing the invasive tree *Ailanthus altissima* and restoring natural cover. Journal of Arboriculture 29: 18–24.

Chen SC, Zhang L, Zeng J, Shi F, Yang H, Mao YR, Fu CX. 2012. Geographic variation of chloroplast DNA in *Platycarya strobilacea* (Juglandaceae). Journal of Systematics and Evolution 50: 374–385.

Clement M, Posada D, Crandall K. 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1660.

Comes HP, Tribsch A, Bittkau C. 2008. Plant speciation in continental island floras as exemplified by *Nigella* in the Aegean Archipelago. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 3083–3096.

Corbett SL, Manchester SR. 2004. Phytogeography and fossil history of *Ailanthus* (Simaroubaceae). International Journal of Plant Science 165: 671–690.

Cun YZ, Wang XQ. 2010. Plant recolonization in the Himalaya from the southeastern Qinghai-Tibetan plateau: Geographical isolation contributed to high population differentiation. Molecular Phylogenetic and Evolution 56: 972–982.

Demesure B, Sodzi N, Petit RJ. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology 4: 129–134.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. Phytochemistry Bull 19: 11–15.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47–50.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Fan DM, Yue JP, Nie ZL, Li ZM, Comes HP, Sun H. 2013. Phylogeography of *Sophora davidii* (Leguminosae) across the 'Tanaka-Kaiyong Line', an important phytogeographic boundary in Southwest China. Molecular Ecology 22: 4270–4288.

Fu YX, Li WH. 1993. Statistical tests of neutrality of mutations. Genetics 133: 693–709.

Gao LM, Möeller M, Zhang XM, Hollingsworth ML, Liu J, Mill RR, Gibby M, Li DZ. 2007. High variation and strong phylogeographic pattern among cpDNA haplotypes in *Taxus wallichiana* (Taxaceae) in China and North Vietnam. Molecular Ecology 16: 4684–4698.

Gong W, Chen C, Dobes C, Fu CX, Koch MA. 2008. Phylogeography of a living fossil, Pleistocene glaciations forced *Ginkgo biloba* L. (Ginkgoaceae) into two refuge areas in China with limited subsequent postglacial expansion. Molecular Phylogenetic and Evolution 48: 1094–1105.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Harrison SP, Yu G, Takahara H, Prentice IC. 2001. Palaeovegetation: Diversity of temperate plants in east Asia. Nature 413: 129–130.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907–913.

Hickerson MJ, Carstens BC, Cavender-Bares J, Crandall KA, Graham CH, Johnson JB, Rissler L, Victoriano PF, Yoder AD. 2010. Phylogeography's past, present and future: 10 years after. Molecular Phylogenetic and Evolution 54: 291–301.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755.

Kowarik I, Säumel I. 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspectives in Plant Ecology 8: 207–237.

Kurokochi H, Saito Y, Chuman M, Ide Y. 2013. Low chloroplast diversity despite phylogenetically divergent haplotypes in Japanese populations of *Ailanthus altissima* (Simaroubaceae). Botany 91: 148–154.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X, Version 2.0. Bioinformatics 23: 2947–2948.

Li EX, Sun Y, Qiu YX, Guo JT, Comes HP, Fu CX. 2008. Phylogeography of two East Asian species in *Croomia* (Stemonaceae) inferred from chloroplast DNA and ISSR fingerprinting variation. Molecular Phylogenetic and Evolution 49: 702–714.

Li GD, Yue LL, Sun H, Qian ZG. 2012. Phylogeography of *Cyananthus delavayi* (Campanulaceae) in Hengduan Mountains inferred from variation in nuclear and chloroplast DNA sequences. Journal of Systematics and Evolution 50: 305–315.

Liu JQ, Sun YS, Ge XJ, Gao LM, Qiu YX. 2012. Phylogeographic studies of plants in China: Advances in the past and directions in the future. Journal of Systematics and Evolution 50: 267–275.

Liu Y, Wang Y, Huang H. 2009. Species-level phylogeographical history of *Myricaria* plants in the mountain ranges of western China and the origin of *M. laxiflora* in the Three Gorges mountain region. Molecular Ecology 18: 2700–2712.

Marr KL, Allen GA, Hebda RJ, McCormick LJ. 2013. Phylogeographical patterns in the widespread arctic-alpine plant *Bistorta vivipara* (Polygonaceae) with emphasis on western North America. Journal of Biogeography 40: 847–856.

Meng HH, Zhang ML. 2013. Diversification of plant species in arid Northwest China: Species-level phylogeographical history of *Lagochilus* Bunge ex Bentham (Lamiaceae). Molecular Phylogenetics and Evolution 68: 398–409.

Meng L, Yang R, Abbott RJ, Miehe G, Hu T, Liu J-Q. 2007. Mitochondrial and chloroplast phylogeography of *Picea crassifolia* (Pinaceae) in the Qinghai–Tibetan Plateau and adjacent highlands. Molecular Ecology 16: 4128–4137.

Millien-Parra V, Jaeger JJ. 1999. Island biogeography of the Japanese terrestrial mammal assemblage: An example of a relict fauna. Journal of Biogeography 26: 959–972.

Nei M, Tajima F. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics 105: 207–217.

Nylander JAA. 2004. MrModeltest v2 [online]. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University. Available from http://www.abc.se/~nylander/ [accessed 19 November 2013].

Ono Y. 1984. Last glacial paleoclimate reconstructed from glacial and periglacial landforms in Japan. Geographical Review of Japan (Series B) 57: 87–100.

Panchal M. 2007. The automation of nested clade phylogeographic analysis. Bioinformatics 23: 509–510.

Posada D, Crandall KA. 2001. Selecting the best-fit model of nucleotide substitution. Systematic Biology 50: 580–601.

Posada D. 2005. Modeltest 3.7 [online]. Available from http://darwin.uvigo.es/ [accessed 28 May 2011].

Provan J, Bennett KD. 2008. Phylogeographic insights into cryptic glacial refugia. Trends in Ecology and Evolution 23: 564–571.

Qiu YX, Fu CX, Comes HP. 2011. Plant molecular phylogeography in China and adjacent regions: Tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. Molecular Phylogenetics and Evolution 59: 225–244.

Qiu YX, Guan BC, Fu CX, Comes HP. 2009a. Did glacials and/or interglacials promote allopatric incipient speciation in East Asian temperate plants? Phylogeographic and coalescent analyses on refugial isolation and divergence in *Dysosma versipellis*. Molecular Phylogenetic and Evolution 51: 281–293.

Qiu YX, Sun Y, Zong M, Zhang XP, Lee J, Murata J, Fu CX, Comes HP. 2009b. Molecular phylogeography of East Asian *Kirengeshoma* in relation to Quaternary climate change and land-bridge configurations. New Phytologist 183: 480–495.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise differences. Molecular Biology and Evolution 9: 552–569.

Ronquist F. 1996. DIVA: Dispersal-vicariance analysis. Version 1.1. Uppsala: Uppsala University.

Rosenberg MS, Anderson CD. 2011. PASSaGE: Pattern analysis, spatial statistics and geographic exegesis. Version 2. Methods in Ecology and Evolution 2: 229–232.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by coalescent and other methods. Bioinformatics 19: 2496–2497.

Sang T, Crawford DJ, Stuessy TF. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). American Journal of Botany 84: 1120–1136.

Shafer ABA, Cullingham C, Côté SD, Coltman DW. 2010. Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America. Molecular Ecology 19: 4589–4621.

Shimono Y, Kurokawa S, Nishida T, Ikeda H, Futagami N. 2013. Phylogeography based on intraspecific sequence variation in chloroplast DNA of *Miscanthus sinensis* (Poaceae), a native pioneer grass in Japan. Botany 91: 449–456.

Swofford DL. 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585–595.

Tian B, Liu RR, Wang LY, Qiu Q, Chen KM, Liu JQ. 2009. Phylogeographic analyses suggest that deciduous species (*Ostryopsis davidiana* Decne., Betulaceae) survived in northern China during the last glacial maximum. Journal of Biogeography 36: 2148–2155.

Tian S, Luo LC, Ge S, Zhang ZY. 2008. Clear genetic structure of *Pinus kwangtungensis* (Pinaceae) revealed by a plastid DNA fragment with a novel minisatellite. Annals of Botany 102: 69–78.

Wang FY, Gong X, Hu CM, Hao G. 2008. Phylogeography of an alpine species *Primula secundiflora* inferred from the chloroplast DNA sequence variation. Journal of Systematics and Evolution 46: 13–22.

Wang J, Gao P, Kang M, Lowe AJ, Huang H. 2009. Refugia within refugia: The case study of a canopy tree *Eurycorymbus cavaleriei* in subtropical China. Journal of Biogeography 36: 2156–2164.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Wu CY. 1979. The regionalization of Chinese flora. Acta Botanica Yunnanica 1: 1–22.

Yang FS, Li YF, Ding X, Wang XQ. 2008. Extensive population expansion of *Pedicularis longiflora* (Orobanchaceae) on the Qinghai-Tibetan Plateau and its correlation with Quaternary climate change. Molecular Ecology 17: 5135–5145.

Yang ZY, Yi TS, Pan YZ, Gong X. 2012. Phylogeography of an alpine plant *Ligularia vellerea* (Asteraceae) in the Hengduan Mountains. Journal of Systematics and Evolution 50: 316–324.

Yesson C, Toomey NH, Culham A. 2009. *Cyclamen*: Time, sea and speciation biogeography using a temporally calibrated phylogeny. Journal of Biogeography 36: 1234–1252.

Zhang HX, Zhang ML. 2012. Identifying a contact zone between two phylogeographic lineages of *Clematis sibirica* (Ranunculeae) in the Tianshan and Altai Mountains. Journal of Systematics and Evolution 50: 295–304.

Zhang TC, Comes HP, Sun H. 2011. Chloroplast phylogeography of *Terminalia franchetii* (Combretaceae) from the eastern Sino-Himalayan region and its correlation with historical river capture events. Molecular Phylogenetics and Evolution 60: 1–12.

Zhao C, Wang CB, Ma XG, Liang QL, He XJ. 2013. Phylogeographic analysis of a temperate-deciduous forest restricted plant (*Bupleurum longiradiatum* Turcz.) reveals two refuge areas in China with subsequent refugial isolation promoting speciation. Molecular Phylogenetics and Evolution 68: 628–643.