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Chloroplast View of the Population Genetics and Phylogeography of a Widely Distributed Shrub Species, *Rhododendron dauricum* (Ericaceae)

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Abstract—Climatic oscillations during the last glacial maximum (LGM) have played important roles in the distributions and genetic structures of extant species. Here, we investigated the population structure of a deciduous shrub species, *Rhododendron dauricum* L., across its current geographic distribution in northeastern China based on six chloroplast regions (*trnS-trnM*, *trnL-trnF*, *rpsC1-rpoB*, *trnS-trnG*, *trnH-psbA*, and *trnK-matK*). A total of 11 haplotypes were identified. The results showed a significant geographic distribution pattern (*Nst* > *Gst*, *p* < 0.01) in northeastern China. In addition, our results showed that there were high levels of genetic diversity in *R. dauricum* and that more than 70% of the genetic variability was distributed among groups. Furthermore, results from the neutrality test failed to identify population expansion at the whole-species level. These findings together suggested that *R. dauricum* shows spatial genetic structure and that the LGM has had profound impacts on the geographic distribution and genetic variability of this species.

Keywords—Genetic diversity, haplotype, last glacial maximum, spatial genetic structure.

The climatic oscillations of the last glacial maximum (LGM, 21–18 ka) have resulted in repeated drastic environmental changes that have profoundly shaped the current distributions and genetic structures of extant species (Hewitt 2000, 2003). Based on population genetics, fossil pollen, and paleoecological evidence, a series of recent studies from diverse plant species have revealed some basic phylogeographic patterns and concepts that have together shed light on the Quaternary history of temperate plants (Kamei 1981; Huntley and Birks 1983; Tsukada 1988; Webb and Bartlein 1992; Lang 1994). One classic view is the southern refugia model (Bennett et al. 1991). According to this model, extensive latitudinal range shifts occurred during the LGM. For example, southward retreats during glacials were followed by rapid expansions northward during inter-/postglacials (Fujii et al. 2002; Griffin and Barrett 2004; Tsuda and Ide 2005; Bartish et al. 2006; de Lafontaine et al. 2010). The repeated contractions and expansions of plant species have shaped plant distribution patterns in the Northern Hemisphere (Hewitt 1996, 1999). Examples from Europe and North America have demonstrated the roles of southern glacial refugia in the formation of current ecosystems (Taberlet et al. 1998; Soltis et al. 2006). However, recent studies of widespread north temperate boreal plants have revealed a different viewpoint, namely that some species survived the LGM in northern refugia near the ice margin (McLachlan et al. 2005; Wall et al. 2010; Beatty and Provan 2011; Nakamura et al. 2014). These studies indicated that in the East Asian temperate forests, phylogeographic patterns cannot be simply explained by southern glacial refugia (Qiu et al. 2011). For example, Opgenoorth et al. (2010) inferred that the *juniperus tibetica* (Kom.) Kom. complex survived the LGM in situ on the south-east edge of the Qinghai-Tibetan Plateau, which had multiple refugia across its range. In addition, Wang et al. (2008) revealed that *Primula secundiflora* Franch. showed multiple separated refugia within a large geographic region without range expansion. Similarly, studies in *Ginkgo biloba* L. (Gong et al. 2008), *Crotonia japonica* Miq., and *C. heterosepala* (Bak.) Oku. (Li et al. 2008) also showed only limited (the former) or even no (the latter two) range expansion; the range expansions during the inter-/postglacials of *Alsophila spinulosa* (Wall. ex Hook.) R. M. Tryon (Su et al. 2005), *Pinus kwangtungensis* Chun ex Tsiang (Tian et al. 2008), *Platycrater arguta* Siebold et Zucc. (Qiu et al. 2009a), *Kirengeshoma kororeana* Nakai, and *K. palmata* Yatabe (Qiu et al. 2009b) were localized. Those studies indicated that some (primarily dominant) species in the temperate mixed conifer and broadleaved forests of East Asia might have survived the glaciations in situ and displayed a limited population expansion during post-glaciations. These species might not have experienced drastic cycles of southward range contraction and northward expansion.

In this study, we investigated the genetic structure of *Rhododendron dauricum* L. (Ericaceae) across its current geographic distribution in northeastern China. *R. dauricum* is a temperate deciduous Northeast Asia endemic shrub species widely distributed in northeastern China, the Korean Peninsula, and the Russian Far East. In northeastern China, *R. dauricum* occurs primarily in the Greater Khingan Mountains (GKM), Lesser Khingan Mountains (LKM), and Changbai Mountains (CM). Characterized by both a temperate climate and a complex topography, these areas have never been directly impacted by extensive and unified ice sheets (Shi et al. 1986; Liu 1988). The Quaternary history of species distributed in this region may display divergent characteristics.

To this end, we used cpDNA sequences to describe the population structure and investigate the phylogeographic history of *R. dauricum* in northeastern China. The aims of this study are as follows: (1) examine the genetic diversity and genetic differentiation among different geographic and spatial genetic groups of *R. dauricum*, (2) identify the intra-species phylogenetic relationships of chloroplast haplotypes and (3) test phylogeographic and demographic patterns in *R. dauricum*. We expect our findings to provide novel evidence for the hypothesis that some taxa persisted in situ in northeastern China during the LGM.

Materials and Methods

Sampling and DNA Extraction—A total of 166 *R. dauricum* individuals were collected from 23 populations that covered its current geographic range in northeastern China (Appendix 1; Fig. 1). In brief, a total of
is also distributed in the
plus), 0.6
1. The four geographic regions of the 23 populations and geo-
μ
and
trnH-psbA
N
JIANG ET AL.: PHYLOGEOGRAPHY OF RHODODENDRON DAURICUM 627
—
F
and
(a measure of genetic differentiation that incorporates phyloge-
—
identified
rpoC1
55 accessions from seven populations were sampled from the GKM;
29 individuals of four populations were collected from the LKM; eight
(GKM, LKM, CM, and LP). To further detect if the studied
populations have experienced expansion, Fu and Li’s D and F (Fu and Li
1993) were calculated for the total population and the four geographic
regions using DnaSP. A spatial analysis of molecular variance (SAMOVA)
was used to analyze the spatial genetic structure of populations using
SAMOVA 1.0 (Dupanloup et al. 2002), with the K varying from 2–20 and
each simulation starting from 100 random initial conditions. To further
assess the partitioning of genetic variation, an analysis of molecular vari-
amo (AMOVA; Excoffier et al. 1992) was performed using Arlequin
3.11 with 1,000 random permutations to test for the significance of partitions,
grouping populations by geographic region.

Phylogenetic and Phylogeographic Analyses—Haplotype identi-
fication and network reconstruction were performed using TCS 1.21
(Clement et al. 2000). The fix connection limit was set as 41 steps when
gaps were treated as missing and 65 steps when gaps were considered a
fifth state to enable all haplotypes to be connected. Phylogenetic relations-
ships among haplotypes were inferred from tests of the Neighbor-Joining
(NJ) method, which were calculated using MEGA 5 (Tamura et al. 2011)
with the Maximum Composite Likelihood method as a substitution model.
We chose 1,000 bootstrap replicates (Felsenstein 1985) to evaluate clade
support. To assess the correlation between genetic and geographic dis-
tance, an isolation-by-distance (IBD) analysis, with 1,000 randomizations
on matrices of pairwise population FST values and the geographic dis-
tances, was performed using Isolation By Distance Web Service Version
3.23 (jensen et al. 2005). To test for the presence of phylogeographic struc-
ture, FST (a measure of genetic differentiation that incorporates phylol-
netic distance) was compared to permuted values of GST (an unordered
measure of genetic differentiation that does not incorporate phyloge-
netic distance) with the program PERMUT (http://www.pierroton.inra.fr/
genetics/labo/Software/PermutcpSSR). Populations containing fewer
than three individuals cannot be incorporated in PERMUT; accordingly, these populations were not included.

Results

cpDNA Diversity and Demographic Analyses—The concatenated cpDNA sequences (including indels) had an aligned length of 4,130 bp, in which 57 SNPs and 11 haplo-
types were identified (Table 1). All new sequence data gener-
ated from this study were submitted to GenBank under the accession numbers KT696608 – KT697615. In all 22 populations analyzed, the haplotype diversity (Hd) and nucleotide di-
versity (π) ranged from 0–0.733 and from 0–0.00594, respectively. The genetic diversity parameters of population BY were the
highest, with the highest values of both Hd and π. In all four geographic regions, the values of haplotype diversity (Hd) and nucleotide diversity (π) ranged from 0.353–0.605 and from 0.0011–0.00269, respectively. LP had the highest genetic diversity.

Analyses of a neutrality test of the complete data set did not support the hypothesis that R. dauricum had undergone a historical population expansion. Neutrality test statistics,
both overall and in the four geographic regions, were non-
significant or significant and positive (Table 1).
TABLE 1. Chloroplast genetic diversity and results of neutrality tests for *R. dauricum*. *S* = number of polymorphic sites (SNPs); *h* = number of haplotypes, *Hd* = haplotype diversity; *π* = nucleotide diversity; NC, not calculated; *p* < 0.02.

<table>
<thead>
<tr>
<th>Group</th>
<th><em>S</em></th>
<th><em>h</em></th>
<th><em>Hd</em></th>
<th><em>π</em></th>
<th>Haplotype</th>
<th>Fu and Li’s <em>D</em></th>
<th>Fu and Li’s <em>F</em></th>
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<tbody>
<tr>
<td>Total</td>
<td>57</td>
<td>11</td>
<td>0.834</td>
<td>0.00238</td>
<td>H1–H11</td>
<td>2.12684</td>
<td>1.39211</td>
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<td>AL</td>
<td>1</td>
<td>2</td>
<td>0.600</td>
<td>0.00015</td>
<td>H1, H2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>AE</td>
<td>1</td>
<td>2</td>
<td>0.533</td>
<td>0.00013</td>
<td>H1, H2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>AK</td>
<td>1</td>
<td>2</td>
<td>0.533</td>
<td>0.00013</td>
<td>H1, H2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>JH</td>
<td>0</td>
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<td>0.000</td>
<td></td>
<td>H1</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>ZL</td>
<td>1</td>
<td>2</td>
<td>0.200</td>
<td>0.00005</td>
<td>H1, H4</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>TP</td>
<td>1</td>
<td>2</td>
<td>0.400</td>
<td>0.00010</td>
<td>H1, H2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>GH</td>
<td>1</td>
<td>2</td>
<td>0.400</td>
<td>0.00010</td>
<td>H1, H2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>HS</td>
<td>0</td>
<td>1</td>
<td>0.000</td>
<td></td>
<td>H2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>HH</td>
<td>0</td>
<td>1</td>
<td>0.000</td>
<td></td>
<td>H2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>ML</td>
<td>3</td>
<td>3</td>
<td>0.378</td>
<td>0.00022</td>
<td>H1, H2, H3</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>TW</td>
<td>0</td>
<td>1</td>
<td>0.000</td>
<td></td>
<td>H2</td>
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<td>NC</td>
</tr>
<tr>
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<td>1</td>
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<td></td>
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<tr>
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<td></td>
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<td>NC</td>
</tr>
<tr>
<td>CB</td>
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<td>1</td>
<td>0.000</td>
<td></td>
<td>H7</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>WT</td>
<td>1</td>
<td>2</td>
<td>0.333</td>
<td>0.00008</td>
<td>H7, H9</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>LS</td>
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<td>0.00044</td>
<td>H5, H7</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>SJ</td>
<td>3</td>
<td>2</td>
<td>0.200</td>
<td>0.00005</td>
<td>H5, H6</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>JD</td>
<td>1</td>
<td>2</td>
<td>0.200</td>
<td>0.00005</td>
<td>H5, H6</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>FH</td>
<td>0</td>
<td>1</td>
<td>0.000</td>
<td></td>
<td>H5</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>BY</td>
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<td>4</td>
<td>0.733</td>
<td>0.00594</td>
<td>H5, H6, H10, H11</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>WL</td>
<td>0</td>
<td>1</td>
<td>0.000</td>
<td></td>
<td>H5</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>GKM</td>
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<td>3</td>
<td>0.446</td>
<td>0.00011</td>
<td>H1, H2, H4</td>
<td>-0.01934</td>
<td>-0.72936</td>
</tr>
<tr>
<td>LKM</td>
<td>3</td>
<td>3</td>
<td>0.463</td>
<td>0.00031</td>
<td>H1, H2, H3</td>
<td>0.95411</td>
<td>1.31094</td>
</tr>
<tr>
<td>CM</td>
<td>6</td>
<td>4</td>
<td>0.605</td>
<td>0.00032</td>
<td>H5, H7, H8, H9</td>
<td>1.17948</td>
<td>0.90646</td>
</tr>
<tr>
<td>LP</td>
<td>45</td>
<td>4</td>
<td>0.353</td>
<td>0.00269</td>
<td>H5, H6, H10, H11</td>
<td>1.68697*</td>
<td>1.33221</td>
</tr>
</tbody>
</table>

Spatial Genetic Analysis, Phylogeographic Structure, and AMOVA—The optimal number of groups indicated by SAMOVA was *K* = 3 (FCT = 0.82673; Appendix 3). Thus, all 23 populations were divided into three groups. Group 1 contained all 11 populations from GKM and LKM, group 2 was composed of populations from CM and LP except for population BY, and group 3 is a single-population group consisting of population BY.

The IBD test revealed a significant correlation between genetic and geographic distances of cpDNA haplotypes over all populations (*r* = 0.5084, *p* < 0.0010) as well as in each main SAMOVA group (*r* = 0.2974, *p* = 0.0270 for group 1 and *r* = 0.5752, *p* < 0.0010 for group 2). *Nst* of *R. dauricum* (0.851) was significantly higher than *Gst* (0.749) at a global level (*p* < 0.01) and the two grouping schemes tested (Table 2), which indicated significant phylogeographic structure in the data set regardless of the grouping scheme used. AMOVA analysis revealed that the main source of variation was found among the groups (Table 2). For example, 72.27% and 82.67% (*p* < 0.01) of the total variation were derived from the geographic and SAMOVA groups, respectively. These findings suggested that *R. dauricum* showed obvious spatial genetic structure in its current distribution range in northeastern China.

Haplotype and Phylogenetic Analyses—The topology obtained from TCS was used to infer relationships among the 11 cpDNA haplotypes (Fig. 2a; Fig. 1), of which H1 (observed 39 times, in 23.49% of individuals), H2 (observed 36 times, 21.69%), H5 (observed 30 times, 18.07%), and H7 (observed 26 times, 15.66%) were the most frequent haplotypes. In addition, the haplotypes H10, H2, H5, and H7 were located in the center of the haplotype network. Moreover, our results also showed that all haplotypes from CM (H5, H7, H8, and H9) and haplotype H6 from LP were clustered together as one clade (Clade I). Similarly, Clade II consisted of H1, H2, H3, and H4, which only occurred in GKM and LKM. The remaining haplotypes (H10 and H11) occurred in LP and were clustered as Clade III. More than half of the populations were fixed for a single haplotype (13 of the 23 populations). Haplotypes H3, H4, and H9 were found to be private alleles of populations ML, ZL, and WT, respectively, whereas haplotypes H10 and H11 were found to be private alleles in population BY. When gaps were set as fifth informative sites (Fig. 2b), there were no significant changes in the haplotype network. Although more haplotypes were identified, they were merely further subdivisions of the haplotypes with gaps regarded as missing.

Phylogenetic relationships of the 11 cpDNA haplotypes were assessed under NJ analyses. All haplotypes were clustered into three clades (Fig. 2c), which was consistent with the haplotype network (Fig. 2a). Haplotypes H5–H9 were clustered as Clade I; Clade II contained H1–H4; H10 and
H11 were clustered as Clade III. The support bootstrap values for internal nodes of the tree were generally high. We checked the segregating sites of these haplotypes. Numbers above branches are bootstrap support values based on 1,000 replicates. Section size of the pie reflects the frequency of individuals from each geographic region.

**Fig. 2.** (a) Statistical parsimony network of 11 cpDNA haplotypes recovered from 166 accessions of _R. dauricum_ with gaps set as missing. Haplotype circle area represents relative frequency. Haplotypes were arbitrarily assigned numbers 1–11 and color-coded to indicate different geographic regions. The dots on branches indicate mutational steps, and the double hatch marks represent the omission of dots. (b) Statistical parsimony network with gaps set as a fifth state. Haplotype numbers were arbitrarily assigned numbers 1-2 for Clade I, 3-4 for Clade II, and 5-6 for Clade III, including indels), which showed quite different nucleotide variation patterns among the three clades.

**Discussion**

**Genetic Diversity and Population Structure**—Of the 73 primer pairs tested in this study, six were amplified successfully and shown to be polymorphic. When confronted with chloroplast marker choice in _Rhododendron_, _Ericaceae_, or other related families, these six primer pairs and associated chloroplast regions may be priorities. From the sequences amplified by those primer pairs, our results showed that the haplotype diversity (\(H_d = 0.834\)) of _R. dauricum_ was higher than that of other temperate shrub or small-sized tree species, such as _R. delavayi_ (C. B. Clarke) Ridley (\(H_d = 0.634\); Sharma et al. 2014), wild _Prunus pseudocerasus_ Lindl. (\(H_d = 0.557\); Shi and Zhang 2015), and the _Rhododendron_ H11 were clustered as Clade III. The support bootstrap values for internal nodes of the tree were generally high. We checked the segregating sites of the 11 cpDNA haplotypes. Numbers above branches are bootstrap support values based on haplotypes in (a). (c) NJ phylogenetic trees for the 11 cpDNA haplotypes. Numbers above branches are bootstrap support values based on 1,000 replicates. Section size of the pie reflects the frequency of individuals from each geographic region.

Of all 23 populations, population BY had the highest genetic diversity (\(H_d = 0.733\); \(\pi = 0.00594\)). Of all four geographic regions, CM had the highest haplotype diversity (\(H_d = 0.605\)) and LP had the highest nucleotide diversity (\(\pi = 0.00269\)). Therefore, the two regions were identified as potential genetic diversity centers of _R. dauricum_. The results of AMOVA showed that the source of most genetic variability was among groups. According to the standard of Wright (1978), the differentiation values calculated among groups (\(F_{CT}\)), populations (\(F_{SC}\)), and individuals (\(F_{ST}\)) were all more than 0.25, indicating a great genetic differentiation of groups on different levels. Similar results were obtained from the SAMOVA cluster, showing that the genetic structures of different clusters were significantly different, while the origins of _R. dauricum_ from different groups were diverse.

The observation of high genetic diversity indicated that _R. dauricum_ might not have undergone a severe genetic bottleneck in the evolutionary process. In contrast, the nucleotide diversity (\(\pi = 0.00238\)) of _R. dauricum_ does not show a high level compared with the haplotype diversity. Our results were similar to previous studies, such as _Kandelia candel_ (L.) Druce (\(\pi = 0.00304\); Chiang et al. 2001), _Fatsia japonica_ (Thunb.) Decne. et Planch. (\(\pi = 0.00285\); Chen 2004), and wild _Prunus pseudocerasus_ (\(\pi = 0.00209\); Chen et al. 2015). To test the factors that have led to the contrasting observation of the high haplotype diversity and the low nucleotide diversity of _R. dauricum_, we checked the segregating sites of these haplotypes. Only 57 SNPs were observed, and the three clades showed quite different nucleotide variation patterns. Accordingly, we consider that the small number of SNPs within each population might not have undergone a severe genetic bottleneck in the evolutionary process. In contrast, the nucleotide diversity (\(\pi = 0.00238\)) of _R. dauricum_ does not show a high level compared with the haplotype diversity.
higher than $G_{ST}$ at the global, geographic, and grouping levels. Moreover, the network and NJ tree of haplotypes showed clear geographic structure. The amazing uniformity of SAMOVA clustering ($K = 3$) and geography further confirmed that populations from different origins had a distinct genetic makeup. Therefore, we propose that the current distribution of *R. dauricum* might result from occupying different refugia during the LGM. For example, populations from the GKM and CM might have had distinct refugia during the LGM. Similar phenomena have also been observed in *Ostryopsis davidiana* Decone. (Tian et al. 2009), *Fagus engleriana* Seemen ex Diels (Lei et al. 2012), *Quercus variabilis* Blume (Chen et al. 2012a) and *Igulagynhysis* Dode (Bai et al. 2014). In addition, the results of the neutrality test suggested stable population dynamics recently, implying no spatial expansions in the post-glaciations. Regarding Fu and Li's *D*, we found that it shows a significant positive value at the species level, possibly due to the population structure observed within this species. Similarly, when all 23 populations or populations within each main SAMOVA group were pooled together, the IBD test showed a significant correlation between genetic and geographic distance. It also indicates that these populations might not have undergone recent population expansion. Ikeda and Setoguchi (2013) used an isolation-with-migration (IM) model to estimate demographic history of *Phylodoce nipponica* Makino. Their results showed that mixing of populations and gene exchange did not emerge in the post-glaciations, so the differences between isolated refugia have not been eliminated. Likewise, our results proved that the genetic imprint from different refugia still deeply influences the genetic structure of *R. dauricum* in northeastern China.

Qian and Ricklefs (2000) and Harrison et al. (2001) proposed two different hypotheses for Asian species diversity. Harrison et al. (2001) suggested that temperate forests would have retreated southward to approximately 30°N during the LGM, so a genetic signature of 'southern richness' and 'northern purity' in many species should be detected. On the contrary, Qian and Ricklefs (2000) observed that there have been multiple isolated refugia for northern China plants possibly due to the great physiographical heterogeneity in this region, providing abundant opportunities for allopatric speciation in the Quaternary. Our results are consistent with the latter view. Habitat fragmentation formed barriers against gene flow, further leading to genetic diversification, accelerating variation and even speciation (DeChaine and Martin 2004; Li et al. 2012; Gao et al. 2015). Under this hypothesis, the long-term isolation among multiple refugia and little admixture among populations of different origins have caused substantial genetic diversification in *R. dauricum*. Given that this may be an example of cryptic speciation, we therefore checked the nucleotide variation patterns of *R. dauricum* and we found that there exist some fixation sites among the three clades. The deep divergence of these clades suggested the possibility of cryptic speciation within this species. However, only a few selected loci were used in this study, which largely limited our inference on this topic. To this end, further investigations based on genome-wide markers might provide novel insights into this issue.

In conclusion, cpDNA haplotypes have delineated the genetic diversity and population genetic structure of *R. dauricum*, providing insights into the phylogeographic patterns. Our results showed high levels of genetic diversity and great genetic differentiation among different groups. A significant phylogeographic structure was also detected in this species. Moreover, the phylogeographic and demographic patterns of multiple localized refugia and no postglacial range expansion have been proved. Our results are consistent with the hypothesis that the complex landforms of East Asia have provided local species with opportunities to survive the last glaciation in situ.

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**Literature Cited**


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![Fig. 5. Segregating sites of the 11 cpDNA haplotypes. All sequences are compared to the reference haplotype H1. The fixation sites in Clade I (H5–H9), Clade II (H1–H4) and Clade III (H10 and H11) are expressed in red, yellow, and blue.](image-url)


to East China and South Japan, inferred from chloroplast DNA sequence variation. Taxon 58: 1226–1241.


Sharma, A. R. C. Poudel, A. Li, J. Xu, and K. Guan. 2014. Genetic diversity study. Data are presented in the following order: population, location, sample size, sampling date, and individual numbers.

Appendix 2. Information about the 73 primer pairs tested in this study. Data are in the following order: chloroplast regions, primer names and sequences, product size (bp), annealing temperature (°C), literature source, and result of PCR amplification. Primers in bold were ultimately chosen.


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Appendix 3. K value and FST value pairs observed in SAMOVA with the K values varying from 2 to 20.

2.07501; 3, 0.82673; 4, 0.83737; 5, 0.84424; 6, 0.84910; 7, 0.84968; 8, 0.85014; 9, 0.85345; 10, 0.85498; 11, 0.85523; 12, 0.85582; 13, 0.85475; 14, 0.85514; 15, 0.85542; 16, 0.85631; 17, 0.85686; 18, 0.85790; 19, 0.85901; 20, 0.86137.