Evolutionary History of *Rhus chinensis* (Anacardiaceae) From the Temperate and Subtropical Zones of China Based on cpDNA and Nuclear DNA Sequences and Ecological Niche Model

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To explore the origin and evolution of local flora and vegetation, we examined the evolutionary history of *Rhus chinensis*, which is widely distributed in China’s temperate and subtropical zones, by sequencing three maternally inherited chloroplast DNAs (cpDNA: \textit{trn}L-\textit{trn}F, \textit{psb}A-\textit{trn}H, and \textit{rbc}L) and the biparentally inherited nuclear DNA (nuDNA: \textit{LEAFY}) from 19 natural populations of *R. chinensis* as well as the ecological niche modeling. In all, 23 chloroplast haplotypes (M1–M23) and 15 nuclear alleles (N1–N15) were detected. The estimation of divergence time showed that the most recent common ancestor dated at 4.2 ± 2.5 million years ago (Mya) from cpDNA, and the initial divergence of genotypes occurred at 4.8 ± 3.6 Mya for the nuDNA. Meanwhile, the multimodality mismatch distribution curves and positive Tajima’s \(D\) values indicated that *R. chinensis* did not experience population expansion after the last glacial maximum. Besides, our study was also consistent with the hypothesis that most refugia in the temperate and subtropical zones of China were in situ during the glaciation.

**Keywords:** *Rhus chinensis*, evolutionary history, DNA sequences, China’s temperate and subtropical zone, ecological niche modeling

**INTRODUCTION**

The Quaternary climate oscillations occurred in the past ca. 2.58 million years ago (Mya) have resulted in several glacial and interglacial cycles in the Northern Hemisphere (Shackleton and Opdyke, 1973). These climatic alterations have left imprints in geographical distributions, population structures, and demographic histories of plant and animal species (Abbott et al., 2000; Avise, 2000; Hewitt, 2004, 2011; Qiu et al., 2011, 2013; Wen et al., 2014, 2016), which can be traced by analyses of genetic variations within and between extant populations (Abbott et al., 2000; Johansen and Latta, 2003; Hewitt, 2004). In Europe and North America, the fossil records of plant species and phyllogeographic analyses had indicated common patterns of geographical range shifts.
that plants retreated southwest and to lower elevations during glacial periods and while recolonized rapidly the northern areas and higher elevations during the interglacial and postglacial periods (Nason et al., 2002; Petit et al., 2003; Stewart et al., 2010; Sakaguchi et al., 2011; Segovia et al., 2012; Voss et al., 2012; Tzedakis et al., 2013; de Lafontaine et al., 2014). While in China, especially the Qinghai-Tibet Plateau (QTP) and adjacent regions, considerable research achievements have also been attained on inferring the Quaternary phylogeographic histories of plant species based on the approach of population genetics (e.g., Zhang et al., 2005, 2015; Meng et al., 2007; Chen K.M. et al., 2008; Yang et al., 2008; Wang et al., 2009; Oppenroth et al., 2010; Xu et al., 2010; Qiu et al., 2011; Zou et al., 2012; Wang G.N. et al., 2014; Wen et al., 2014; Liu Y.P. et al., 2015; Wan et al., 2016).

The temperate and subtropical region of China is a model area for studying plant species in response to past climate changes (Chen S.C. et al., 2012; Li X.H. et al., 2012; Qi et al., 2012; Zhao et al., 2013; Fu et al., 2014). Up to date, many phylogeographic studies have been used to elucidate the impacts of the uplifts of the QTP on the climate within the modern-day temperate and subtropical zones, or warm temperate zones in China (e.g., Liu J.Q. et al., 2014; Lu et al., 2016; QTP, Liu D. 2014; Y angtze River, Sun et al., 2013; Wang H. et al., 2015; Qinling Mountains, Liu J.Q. et al., 2014; Lu et al., 2016; QTP, Liu D. et al., 2015 and Liu Y.P. et al., 2015); i.e., 23.5◦N and 98.0◦-124.0◦E. These results showed that the QTP acted as a barrier against glaciation within the warm temperate zones of China and resulted in the arid climate for thousands of years within the Quaternary period, which has been widely accepted nowadays (Wang et al., 2013; Yu et al., 2013; Meng et al., 2014). Thus, the present warm temperate region probably served as a glacial refugia for plant species in the past time, and this hypothesis has been tested and advanced through phylogeographic studies (e.g., Yi et al., 2012; Liu et al., 2012; Qi et al., 2012; Wan et al., 2016). However, it is less well known whether population genetic diversification of plants within the warm temperate zone or within the glacial refugia is due to isolation on a heterogeneous landscape or adaptation and selection along ecological gradients (Su et al., 2015; Zhao et al., 2016). Therefore, more phylogeographic studies of additional plant species within the warm temperate refugial regions are necessary in order to detect their spatial geographic patterns and to assess the underlying causes.

*Rhus chinensis* belongs to the plant family Anacardiaceae and is a common deciduous tree that is endemic to the warm temperate zone of Asia. It widely occurs at the elevation of 170–2700 m above sea level in Shaanxi, Shanxi, Hebei, Sichuan, Hunnan, and Yunnan of China (Zheng and Min, 1980). Due to its commonality and widespread distribution within the warm temperate zone, *R. chinensis* is thus an ideal study case for phytogeography within this region. In this study, we used three cpDNA regions (*trnL-trnF*, *psbA-trnH*, and *rbcL*) and one nuDNA region (*LEAFY*) to examine (1) the genetic diversity and structure of *R. chinensis* populations in China and (2) how is the demographic history of *R. chinensis* during the Quaternary climate oscillations, and further to explore the origin and evolution of local flora and vegetation.

**MATERIALS AND METHODS**

**Population Sampling**

In total, leaf samples of 312 individuals were collected from 19 natural populations of *R. chinensis*, representing its whole geographic distribution within the warm temperate zone of China (see Figure 1 and Table 1). Eight to 20 individuals were collected for each population, and all individuals were at least 15 m apart. We obtained several voucher specimens for each population, which were deposited at the School of Life Sciences, Shanxi University, Taiyuan, Shanxi, China. The information of latitude, longitude, and altitude of each population were recorded using an Etrex GIS (Garmin, Taiwan, China).

The species from Anacardiaceae were used as outgroups. The cpDNA sequences of four species were downloaded from GenBank, *Rhus virens* (EF682861, KF664327, KF664558), *Rhus typhina* (AY640446, HQ427036, HQ590236), *Rhus glabra* (AY640440, KF664325, KX397919), and *Pistacia vera* (EF193139, KF664307, AJ235786). There are no nuclear sequences for the above four species in GenBank, so we used another two species *Mangifera indica* (GU338039) and *P. chinensis* (KC174710) as the outgroups in the nuDNA analysis.

**DNA Sequencing**

Total genomic DNAs from approximately 20 mg of silica gel-dried leaf materials were extracted using a Plant Genomic DNA kit (Tiangen Biotech, Beijing, China), and three cpDNA fragments (*trnL-trnF*, *psbA-trnH*, and *rbcL*) and one nuclear gene (*LEAFY*) were amplified and sequenced by the following primers: *trnL-trnF* (5′-CGAAATCGGTAAGGCGTAC-3′; 5′-ATTGAACTGGTACACAGG-3′; Taberlet et al., 1991), *psbA-trnH* (5′-GTATTGCAATFAACGTAATGCTC-3′; 5′-GGGCGAGTGTGATTCCAACAATC-3′; Sang et al., 1997), *rbcL* (5′-ATGTCACACACACATGAGAC-3′; 5′-TGAATTCACATCGCAGAGATGCTC-3′; Little and Barrington, 2003), *LEAFY* (5′-TACACGGCGAGGAAGATAGC-3′; 5′-CTAGAAGACGGCCGCTATG-3′; Oh and Potter, 2003). Polymerase chain reaction (PCR) was performed in a volume of 50 µL and each reaction contained 30–50 ng genomic DNA, 25 µL amplification reaction mixture (PCR mix kit, Tiangen Biotech, Beijing, China), and 20 µmol/L primers, and under the following conditions: initial denaturation at 94°C for 3 min, 35 cycles of 30 s at 94°C, 30 s at 54–60°C, 90 s at 72°C, and a final extension step of 7 min at 72°C. All the qualified PCR products were sent to Majorbio Bio-pharm Technology Co., Ltd. (Shanghai) for sequencing.

**Data Analysis**

We aligned sequences with Clustal_X (Thompson et al., 1997) and coded indels following the method of Simmons and Ochoterena (2000). Indels within mononucleotide repeat regions were deleted for phylogenetic analyses, because the homology of these indels could not be verified (Chen S.C. et al., 2012).
The levels of inter- and intra-population genetic diversity ($h$: haplotype diversity and $\pi$: nucleotide diversity) were calculated for the cpDNA and nuDNA using DnaSP version 5.0 (Rozas et al., 2003). We compared $G_{ST}$ and $N_{ST}$ using the U-statistic, which is approximated by a Gaussian variable by taking into account the covariance between $G_{ST}$ and $N_{ST}$, and a one-sided test (Pons and Petit, 1996). The former considers only haplotype frequencies while $N_{ST}$ also takes into account differences between haplotypes. When $N_{ST}$ is larger than $G_{ST}$, phylogeographic structure is obvious, which indicates that closely related haplotypes were found more often in the same area than less closely related haplotypes (Pons and Petit, 1996). We also estimated genetic differentiation among all populations with AMOVA and inferred population growth and expansion according to Tajima’s $D$ using Arlequin version 3.0 (Excoffier et al., 2005), with 1000 random permutations to test for significance of partitions. Genealogical relationships among cpDNA and nuDNA haplotypes were constructed using TCS version 1.21 (Clement et al., 2000).

The phylogenetic relationships among haplotypes and genotypes of cpDNA and nuDNA were reconstructed with Bayesian inference (BI) methods in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). We applied the best fit
TABLE 2 | The total haplotype and nucleotide diversity of Rhus chinensis.

| Population | H  | π × 10^{-3} | Haplotype (No.) | H  | π × 10^{-3} | Genotype (No.) |
|------------|----|-------------|----------------|----|-------------|----------------|
| P1         | 0.000 | 0.000 | M1(20) | 0.616 | 1.140 | N1(8), N2(1), N3(10), N4(1) |
| P2         | 0.719 | 8.810 | M1(5), M2(10), M3(2), M4(3), M8(4), M21(2) | 0.686 | 3.870 | N1(2), N3(2), N5(4), N7(1), N15(11) |
| P3         | 0.550 | 5.230 | M5(7), M6(4), M7(2) | 0.422 | 2.270 | N3(10), N4(7) |
| P4         | 0.586 | 5.370 | M2(4), M3(4), M4(7) | 0.505 | 2.570 | N1(4), N3(6), N4(5) |
| P5         | 0.358 | 5.620 | M1(16), M2(2), M9(2) | 0.379 | 1.410 | N3(13), N5(7) |
| P6         | 0.591 | 5.400 | M1(8), M10(4), M11(2) | 0.485 | 2.140 | N1(4), N3(2), N7(5) |
| P7         | 0.571 | 3.520 | M1(5), M10(4), M11(2) | 0.314 | 2.120 | N3(4), N10(2) |
| P8         | 0.526 | 2.590 | M12(4), M13(4) | 0.653 | 2.180 | N3(4), N6(3), N11(8), N12(8) |
| P9         | 0.589 | 5.700 | M5(4), M16(4), M17(12) | 0.605 | 2.580 | N3(1), N4(7), N6(1), N14(5) |
| P10        | 0.400 | 4.970 | M12(3), M13(6), M18(1) | 0.389 | 2.980 | N3(8), N9(2), N10(2) |
| P11        | 0.636 | 8.810 | M1(5), M10(4), M11(2) | 0.321 | 2.540 | N3(1), N4(4), N7(9) |
| P12        | 0.533 | 2.730 | M19(9), M20(7) | 0.000 | 0.000 | N3(13), N4(2) |
| P13        | 0.689 | 6.320 | M1(5), M2(7), M19(8) | 0.300 | 2.150 | N3(4), N6(3), N14(11) |
| P14        | 0.554 | 5.690 | M1(8), M5(5), M6(6), M23(5) | 0.395 | 1.740 | N3(15), N4(5) |
| P15        | 0.694 | 8.220 | M1(5), M6(1), M17(6), M18(2) | 0.221 | 0.970 | N3(15), N4(2) |
| P16        | 0.645 | 8.700 | M1(2), M6(3), M8(2), M21(3), M23(8) | 0.774 | 3.000 | N3(4), N6(3), N14(11) |
| P17        | 0.442 | 2.260 | M19(6), M20(14) | 0.395 | 1.740 | N3(15), N4(5) |
| P18        | 0.318 | 2.510 | M1(2), M22(2), M23(5) | 0.268 | 1.400 | N3(10), N14(2) |
| P19        | 0.689 | 7.820 | M1(4), M2(5), M4(4), M21(2) | 0.281 | 1.830 | N3(12), N7(3) |
| Total      | 0.758 | 6.910 | 0.614 | 3.050 |

π: nucleotide diversity, h: haplotype diversity.

TABLE 3 | Analysis of molecular variance (AMOVA) of Rhus chinensis populations based on nucleotide sequences.

| Gene types | Source of variation | d.f. | SS | VC | PV | F_{ST} |
|------------|---------------------|------|----|----|----|-------|
| Chlorotype | Among regions       | 4    | 1425.914 | 4.95009 | 30.05 |
|            | Among populations   | 14   | 1396.234 | 5.58051 | 33.88 |
|            | Within populations  | 293  | 1740.808 | 5.94132 | 36.07 |
| Genotype   | Total               | 311  | 4562.955 | 16.47192 |       |
| Genotype   | Among regions       | 3    | 321.790 | 1.02156 | 29.33 |
|            | Among populations   | 15   | 146.323 | 0.58156 | 14.59 |
|            | Within populations  | 293  | 427.270 | 1.95386 | 56.09 |
| Genotype   | Total               | 311  | 895.383 | 3.41857 |       |

Notes: d.f., degrees of freedom; SS, sum of squares; VC, variance components; F_{ST}, correlation within populations relative to total; *P < 0.01.

Ecological Niche Modeling
We compared the current distributions of R. chinensis with its inferred distributions during the last glacial maximum (LGM; ~21,000 years BP) with ecological niche modeling in Maxent version 3.3.3 (Phillips et al., 2006). To perform this modeling, we first obtained the geocoordinates of 73 occurrence data of R. chinensis from the Chinese Virtual Herbarium1 and Global Biodiversity Information Facility2. Subsequently, we constructed the models using 19 bioclimatic variables from

1http://www.cvh.org.cn
2http://data.gbif.org
the WorldClim database3 (Hijmans et al., 2005) representing the present (averaged from 1950) and the LGM according to the Community Climate System Model (CCSM; Collins et al., 2006). We employed 20 replicates based on 80% of the distribution coordinates for training and 20% for testing, and adopted the model with the best AUC values (Phillips et al., 2006). We performed a jackknife test to estimate the percent contributions of bioclimatic variables to the prediction for the distributional models. Meanwhile, we also employed the “10 percentile presence” threshold logistic approach as determined by Maxent in order to distinguish the threshold between suitable and unsuitable habitats for further analyses. We drew Graphics for each predicted SDM using DIVA-GIS 7.5 (Hijmans et al., 2005).

RESULTS

Genetic Diversity and Structure

Aligned cpDNA dataset consisted of 2051 bp with 70 nucleotide substitutions and two indels. We detected 23 different haplotypes (M1–M23) based on combined cpDNA dataset from 19 populations. The LEAFY gene region varied from 412 to 645 bp and had an aligned length of 682 bp, which contained 14 nucleotide substitutions. Our sequences of LEAFY comprised 15 genotypes (N1–N15). Based on cpDNA and nuDNA sequences, the total haplotype diversity of R. chinensis was estimated to be 0.738 and 0.614, and the total nucleotide diversity was inferred to be 6.910 × 10−3 and 3.050 × 10−3, respectively (Table 2). We found the highest levels of haplotype and nucleotide diversity in four populations: P2, P11, P14, and P16 (Table 2). The most widespread haplotypes and genotypes were M1 (in 11 of 19 populations, cpDNA) and N3 (in 18 of 19 populations, nuDNA; Table 2), respectively. Based on cpDNA and nuDNA sequences, M1 and N3 were the primary haplotype and genotype, respectively (Figure 1).

AMOVA analysis indicated that genetic variation in R. chinensis was greater within populations than among them (P < 0.01; Table 3). The mismatch distribution (Figures 1A1,B1) and positive values of Tajima’s D value (1.19, 0.05 < P < 0.10 for cpDNA; 2.37, P < 0.01 for nuDNA) of all populations rejected a sudden expansion model, and positive Tajima’s D may indicate population admixture. Phylogeographic structure is not obvious at the species level for both sets of genetic markers. For the cpDNA data, NST (0.382) was slightly higher than GST (0.375),

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3http://www.worldclim.org/
Differences in gametophyte and sporophyte persistence among populations may also be responsible for high levels of gene flow in many other plant species (e.g., Lopez et al., 2007; Song et al., 2013; Johnson et al., 2017). *R. chinensis* may also be responsible for high levels of gene flow among populations. Stated another way, there may be limited barriers to dispersal. In the distributional area of *R. chinensis*, no obvious geographic barriers have been observed. Therefore, *R. chinensis* does not appear to be geographically isolated, allowing ecological niche modeling to be used in the assessment of species status (Li X.H. et al., 2012; Liu L. et al., 2014; Wang W. et al., 2014). Ecological niche models suggested the suitable habitats of *R. chinensis* were continuous in the present time while compressed during the LGM period, demonstrating multiple possible isolated glacial refugia (Figure 3). The response to impact of cold and warm times on the distribution of *R. chinensis* was validated in the simulation of ecological niche modeling, although we only used the simulated environment of current and LGM period (Figure 3). This pattern of range

**DISCUSSION**

We did not detect a clear phylogeographic structure among the 19 populations of *R. chinensis* sampled in the present study. We found a somewhat lower differentiation among *R. chinensis* populations ($N_{ST} = 0.382$ for cpDNA, $N_{ST} = 0.321$ for nuDNA) compared to sympatric species such as *Platyacarya strobilacea* (Chen S.C. et al., 2012) and *Cotinus coggygria* (Wang W. et al., 2014). Limited phylogeographic structure within a metapopulation may be due to high levels of geneflow and/or of geophysical connectedness (Avise et al., 1987). High levels of gene flow among *R. chinensis* populations may be due to the seed dispersal mechanism, which has been implicated in high levels of gene flow in many other plant species (e.g., Lopez et al., 2007; Song et al., 2013; Johnson et al., 2017).
shifts indicated a likely scenario of repeated glacial compressions followed by interglacial expansions for *R. chinensis* during the Quaternary climatic oscillations. It is interesting that the geographic distribution of the cpDNA haplotypes differs from the nuDNA genotypes (Figure 2). Mismatch distributions between organelar DNA haplotypes and nuclear DNA genotypes have been reported in other groups such as *Sophora davidii* (Fan et al., 2013), *Cycas diannanensis* (Liu J. et al., 2015), and *Osteomeles schwerinae* (Wang Z.W. et al., 2015). Therefore, we thought that the forest birds and mammals were known as seed dispersers for many species in Anacardiaceae (Wang W. et al., 2014), which might have directly impacted the genetic structure with biparental inheritance.

The populations originated from Yunnan occurred at the China–Vietnam border and split from other clades at 4.2 ± 2.5 and 3.8 ± 3.0 Ma according to the cpDNA (clade VI) and nuDNA (clade III), respectively (Figures 2A,B). Early diverging populations in Yunnan have been detected in other genera or species such as *Ceratotropis* (3.62 Ma, Javadi et al., 2011), *Incarvillea sinensis* (4.4 Ma, Chen S. et al., 2012), and *Stuckenia filiformis* (3.93 Ma, Du and Wang, 2016). Within these species, the uplift of the QTP has been implicated as the main mechanisms of driving diversifications, but the estimated divergences were more recent than the last phase of the uplift (7–8 and 13–15 Ma; Harrison et al., 1992; Shi et al., 1998; Spicer et al., 2003). So, we thought that the geographical isolation of Yunnan populations was caused by the isolation of the QTP uplift in late Pliocene. Furthermore, the suitable climate in the temperate and subtropical zone could have subsequently facilitated the Pliocene-Pleistocene diversification of *R. chinensis* into different eco-geographic populations (Javadi et al., 2011).

Previous phylogeographic studies have widely supported hypotheses that climatic changes during the LGM forced plants into refugia within Central China, where they were protected by the QTP from the brunt of the ice age (Tian et al., 2009; Liu et al., 2012). After the glaciers retreated, the plants expanded their ranges rapidly (Hewitt, 2000; Li Z.H. et al., 2012; Qi et al., 2012). Our results showed that the range of *R. chinensis* had increased since the LGM (Figure 3) but did not support a rapid expansion based on the mismatch distribution (Figure 1) and Tajima's D (1.19, 0.05 < P < 0.10 for cpDNA; 2.37, P < 0.01 for nuDNA). Refugia in the warm temperate China may have been dominated by evergreen forest or temperate deciduous forest during the LGM (Liu, 1988). Thus, southern Shaanxi, northern Sichuan, Yunnan, and Jiangxi could have supported *R. chinensis* during the LGM and been its main center of diversity. Just as *P. strobilacea* (Chen S.C. et al., 2012), *Cercidiphyllum* (Qi et al., 2012), and *C. coggynria* (Wang W. et al., 2014), the plants were slightly affected and were able to survive in situ at the period of the glaciation. So, the characterized phylogeographic structure of *R. chinensis* was consistent with the second hypothesis, which was that they survived in situ and occupied multiple localized glacial refugia during the glaciation.

**CONCLUSION**

We used cpDNA and nuDNA sequences, and ecological niche modeling to investigate the evolutionary history of *R. chinensis* distributed in the warm temperate zone of China. The cpDNA and nuDNA data separately revealed six and five clades corresponding to the geographic regions. The divergence among haplotypes and genotypes of *R. chinensis* occurred at the Pliocene based on cpDNA and nuDNA data. Our ENMs showed enlarged potential distributions in the present compared to LGM, but we did not detect a sudden demographic expansion after the glaciation according to the molecular data. Our results suggest that *R. chinensis* was not affected by glacial cycles seriously and survived in situ and occupied a few main refugia.

**AUTHOR CONTRIBUTIONS**

ZR conceived and designed the research. YL and YZ collected the samples, performed the experiments, and conducted data analyses. XS and ZR drafted the manuscript. JW polished the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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