Postprint: Genetic Diversity and Genetic Structure of Wild Pinellia ternata in China

Authors: Wu Min, Wu Shiqi, Pan Feng, Shi Tian, Zhao Cai

Date: 2023-08-25T00:00:00+00:00

Abstract

Quaternary climatic fluctuations and geographic and environmental isolation have profoundly influenced the genetic diversity, genetic structure, and geographical distribution patterns of modern plants. This study employed molecular phylogeographic approaches to analyze three chloroplast fragments (psbK-psbI, atpF-atpH, and trnL-F) in 212 individuals from 19 populations of the medicinal plant Pinellia ternata, aiming to investigate its genetic diversity, genetic structure, patterns and causes of geographical distribution, and population historical dynamics. The results showed: (1) The total haplotype diversity Hd of Pinellia ternata was 0.882, and the total nucleotide diversity π was 1.23×10^{-3}, indicating high genetic diversity at the species level. (2) Analysis of Molecular Variance (AMOVA) revealed that genetic variation in Pinellia ternata occurred primarily among populations, with significant genetic differentiation ($FST = 0.909$, $P < 0.001$) and low within-population genetic diversity ($HS = 0.134$); the coefficient of genetic differentiation among populations $NST = 0.913$ was greater than $GST = 0.855$ ($0.01 < P < 0.05$), indicating a distinct phylogeographic structure of chloroplast haplotypes. (3) Neutrality test results showed that Tajima' s D, Fu & Li' s D, and Fu and Li' s F * values were all non-significant positive values, Fu' s Fs value was a non-significant negative value, and the mismatch distribution curve was bimodal, indicating that Pinellia ternata populations as a whole have not experienced expansion events. (4) The geographical distribution of haplotypes revealed that southwestern and central-eastern regions exhibited higher haplotype diversity and contained endemic haplotypes, suggesting the existence of glacial refugia in these two regions during the Quaternary glacial periods. In summary, through the analysis of three chloroplast genes in Pinellia ternata from different regions, this study elucidated its genetic diversity, genetic structure, and geographical distribution patterns, and also provided scientific recommendations and conservation strategies for the molecular screening and protection of elite germplasm of Pinellia ternata.

Full Text

Preamble

Genetic Diversity and Genetic Structure of Wild *Pinellia ternata* (Araceae) in China

Authors: WU Min, WU Shiqi, PAN Feng, SHI Tian, ZHAO Cai*
Affiliation: Key Laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Collaborative Innovation Center for Mountain Ecology & Agro-Bioengineering (CICMEAB), College of Life Sciences/Institute of Agro-bioengineering, Guizhou University, Guiyang 550025, China

Abstract: Quaternary climate fluctuations and geographic-environmental isolation have profoundly influenced the genetic diversity, genetic structure, and geographic distribution patterns of modern plants. This study employed phylogeographic methods to analyze three chloroplast fragments (psbK-psbI, atpF-atpH, and trnL-F) from 212 individuals across 19 populations of the medicinal plant *Pinellia ternata*. We investigated its genetic diversity, genetic structure, geographic distribution patterns and their underlying causes, and explored population historical dynamics. The results revealed: (1) Total haplotype diversity (Hd) of 0.882 and total nucleotide diversity (π) of 1.23×10^{-3}, indicating high genetic diversity at the species level. (2) Analysis of Molecular Variance (AMOVA) showed that genetic variation occurred primarily among populations, with significant genetic differentiation (FST = 0.909, $P < 0.001$) and low within-population genetic diversity (HS = 0.134). The genetic differentiation coefficient among populations (NST = 0.913 > GST = 0.855, $0.01 < P < 0.05$) demonstrated a clear phylogeographic structure of chloroplast haplotypes. (3) Neutrality tests showed non-significant positive values for Tajima' s D, Fu & Li' s D, and Fu and Li' s F*, and a non-significant negative Fu' s Fs value, with a bimodal mismatch distribution curve, indicating that* P. ternata* populations have not experienced expansion events. (4) Haplotype geographic distribution revealed higher haplotype diversity and the presence of private haplotypes in southwestern and central-eastern China, suggesting these regions served as glacial refugia during the Quaternary ice ages. In summary, analysis of three chloroplast genes across different regions of *P. ternata* clarifies its genetic diversity, genetic structure, and geographic distribution patterns, providing scientific recommendations and conservation strategies for molecular screening and protection of elite germplasm.

Keywords: *Pinellia ternata*, geographical distribution pattern, genetic diversity, genetic structure, glacial refugia

Introduction

Global climate change, particularly Quaternary climate fluctuations and environmental changes, has profoundly impacted the genetic diversity, genetic structure, and geographic distribution patterns of modern plants (Hewitt, 1996, 2004; Bennett & Provan, 2008). Phylogeographic studies have been extensively conducted in Europe and North America, revealing the genetic structure and evolutionary history of extant plant populations (Hickerson et al., 2010; Qiu et al., 2011; Liu et al., 2012; Ye et al., 2017). During glacial periods, extensive continental glaciers forced thermophilic plants at high latitudes to retreat southward, forming "glacial refugia" (Hewitt, 2004). Conversely, during interglacial or postglacial periods of warming climate and glacial retreat, plants from refugia migrated back to higher latitudes (Hewitt, 1996, 2004; Shafer et al., 2010). However, the impacts of global climate change and environmental fluctuations on genetic diversity, genetic structure, phylogeographic distribution patterns, and population dynamic history of Chinese plants differ markedly from those in Europe and North America (Qiu et al., 2011; Liu et al., 2012; Meng et al., 2015; Wang et al., 2017), manifesting in several aspects: (1) Due to the absence of extensive ice sheet coverage in China, plants underwent range contractions during glacial periods, with multiple glacial refugia existing; (2) During interglacial or postglacial periods, individuals surviving in refugia experienced range expansions; (3) The uplift of the Qinghai-Tibet Plateau and associated mountain-river isolation, monsoon-induced dry-wet alternations, aridity, and Quaternary glacial-interglacial cycles constitute major factors shaping plant phylogeographic patterns. However, these studies have primarily focused on the Qinghai-Tibet Plateau, southwestern China, the Qinling Mountains and adjacent regions, and have mainly targeted woody plants, with relatively few similar studies on other regions and herbaceous species.

Plant chloroplast DNA (cpDNA) is a circular double-stranded DNA molecule. Compared with nuclear genomic DNA, cpDNA is characterized by small molecular weight, multiple copies, and simple structure, with rare recombination among different cpDNA fragments. Consequently, it exhibits higher genetic differentiation and lower gene flow in plant population studies, enabling clearer elucidation of species' genetic variation and phylogeographic patterns (Avise et al., 2000; Sudhir et al., 2016). Therefore, increasing research has employed multiple chloroplast genes to investigate plant genetic diversity, genetic structure, phylogeographic distribution patterns, and population historical dynamics (Zhang, 2022).

Pinellia ternata (Araceae) is a perennial herbaceous plant, commonly known as Shoutian, Diwen, Matouyu, Tianluoxing, or Wuxincai. As a clinically important traditional Chinese medicine, it grows in moist, warm, shaded, and loose sandy soils below 2,500 m elevation and is endemic to East Asia. Wild *P. ternata* is distributed across all Chinese provinces except Inner Mongolia, Qinghai, Xinjiang, and Tibet (Li et al., 2004; Zhang, 2007). Geographic distance leads to population and phenotypic differentiation over time, affecting its genetic diversity and genetic structure. Meanwhile, extensive commercial exploitation and lack of conservation measures have caused a sharp decline in wild resources, necessitating collection and genetic background analysis. As utilization of *P. ternata* expands, research has proliferated, though primarily focusing on chemical composition, toxicity, and pharmacological effects (Li et al., 2021).

Few scholars have investigated phylogenetic relationships and evolutionary affinities. For instance, Zhao & Li (2016) used matK+rbcL combined sequences to analyze phylogenetic relationships within *Pinellia*, demonstrating that matK+rbcL can serve as DNA barcodes for species identification. Zhang (2007) used ITS sequences to analyze sequence variation among Chinese *P. ternata* populations and its correlation with geographic distribution and morphology, showing that rDNA variation relates to geographic distribution. Pan et al. (2021) used ITS sequences to reveal population genetic structure and diversity. Zhang et al. (2021) developed SSR primers to analyze genetic diversity among populations. Zheng et al. (2013) analyzed cpDNA non-coding regions (psbK-psbI and atpF-atpH) in *P. ternata* and related species, identifying abundant variation sites, yet comprehensive studies on genetic diversity, genetic structure, phylogeographic patterns, and population historical dynamics based on multiple chloroplast genes remain lacking. As a widespread herbaceous species with strong ecological adaptability spanning different climate zones and multiple biodiversity hotspots, *P. ternata* represents an ideal material for investigating genetic structure, phylogeographic patterns, and population historical dynamics in herbs and widespread species.

This study employs phylogeographic methods based on three chloroplast genes (psbK-psbI, atpF-atpH, and trnL-F) to analyze 212 wild samples from 19 natural populations across 15 provinces in eastern, central, northwestern, and southwestern China. We investigate genetic diversity, genetic structure, phylogeographic patterns, and population historical dynamics of this medicinal plant, aiming to reveal genetic variation among and within populations, haplotype geographic distribution patterns, infer glacial refugia during Quaternary ice ages, and propose scientific recommendations and conservation strategies for molecular screening and protection of elite germplasm. This research provides important references for *P. ternata* resource conservation, utilization, and molecular-assisted breeding, while offering theoretical foundations for further exploring Chinese flora evolution and species diversity formation.

1.1 Experimental Materials

Pinellia ternata materials were collected and preserved from 2015 to 2020, covering 15 provinces including Guizhou, Zhejiang, Gansu, and Sichuan, spanning northern and southern China and representing natural geographic distribution zones in eastern, central, northwestern, and southwestern regions. A total of 212 individuals from 19 natural populations were collected, with location in-formation measured using Global Positioning System (Table 1). Fresh leaves were immediately dried with silica gel for total DNA extraction. Voucher specimens are deposited in the Herbarium of the College of Life Sciences, Guizhou University.

1.2 DNA Extraction and Amplification Sequencing

Total DNA was extracted from silica gel-dried leaves using a novel plant genomic DNA extraction kit (column type). Primers were selected based on published *P. ternata* sequences and related reports (Zheng et al., 2013), targeting three chloroplast gene fragments (psbK-psbI, atpF-atpH, and trnL-F), with primers synthesized by Sangon Biotech (Shanghai) Co., Ltd. Primer sequences and PCR amplification protocols are listed in Table 2 . The PCR reaction system (25 L) contained 12.5 L 2× Taq PCR MasterMix, 8.5 L ddH$_2$O, 1 L forward primer, 1 L reverse primer, and 2 L DNA template. Total DNA and PCR products were detected by 1% agarose gel electrophoresis. After electrophoresis, gels were visualized using a gel imaging system, and qualified products were sent to Sangon Biotech (Shanghai) Co., Ltd. for purification and sequencing.

1.3 Data Analysis

Sequences were aligned using MEGA7.0 software (Sudhir et al., 2016) to correct erroneous bases, perform multiple sequence alignment, and manually trim primer regions from both ends. Sequences were assembled using PhyloSuite software (Zhang et al., 2020). DNASP6.0 software was used to count haplotype numbers and calculate haplotype diversity (Hd), nucleotide diversity (π), Tajima' s D, Fu and Li' s F*, Fu & Li' s D, and Fu' s Fs values, with mismatch analysis performed to detect expansion events (Tajima, 1989; Fu, 1997). PERMUTCpSSR 2.0 was used to calculate total genetic diversity (HT), average within-population genetic diversity (HS), and differentiation coefficients (GST and NST), with Network software used to construct haplotype networks (Bandelt et al., 1999). Arlequin software (Lu, 2018) was employed for AMOVA to calculate population genetic structure and detect variation among and within populations, with fixation index (FST) and gene flow (Nm) calculated to reveal population differentiation. ArcGIS 10.2 software was used to map haplotype geographic distributions.

2.1 Sequence Variation and Haplotype Diversity

The combined psbK-psbI+atpF-atpH+trnL-F sequences were successfully obtained from 212 individuals. The aligned sequence length was 1,947 bp, containing 11 variable sites with G + C content of 31.80%. These sequences were submitted to GenBank under accession numbers OL310546-OL310559, OL310532-OL310545, and OL310560-OL310573. As shown in Table 3 , only populations from Zhaotong City, Yunnan Province (C6) in southwestern China; Guiyang City, Guizhou Province (C14); Mianyang City, Sichuan Province (C17); Chizhou City, Anhui Province (C7) in eastern China; and Yiwu City, Zhejiang Province (C13) contained multiple haplotypes, while all other populations had only one haplotype. DNASP analysis revealed total haplotype diversity (Hd) of 0.882 and total nucleotide diversity (π) of 1.23×10^{-3}. Zhaotong City, Yunnan (C6) and Mianyang City, Sichuan (C17) showed the highest haplotype diversity (0.5333), followed by Chizhou City, Anhui (C7) and Yiwu City, Zhejiang (C13). Overall, populations in southwestern and eastern China exhibited higher genetic diversity levels.

2.2 Haplotype Distribution

DNASP analysis of the psbK-psbI+atpF-atpH+trnL-F combined sequences (excluding insertions and deletions) identified 14 haplotypes (H1-H14). Haplotype H10 comprised 53 individuals with the highest frequency. Haplotypes H2 and H10 showed the widest distribution ranges: H10 occurred in Yichang City, Hubei (C2); Qiandongnan, Guizhou (C3); Tianshui City, Gansu (C4); Zhaotong City, Yunnan (C6); and Chizhou City, Anhui (C7), while H2 occurred in Yiwu City, Zhejiang (C13); Shangluo City, Shaanxi (C15); Shijiazhuang City, Hebei (C16); Mianyang City, Sichuan (C17); and Shangrao City, Jiangxi (C19). Except for H1, H2, H10, and H11, which occurred in multiple populations, all other haplotypes were private: H3 only in Yiwu (C13); H4 and H5 only in Guiyang (C14); H6 only in Mianyang (C17); H7 only in Dandong, Liaoning (C1); H8 only in Qingdao, Shandong (C10); H9 only in Guyuan, Ningxia (C18); H12 only in Chizhou (C7); H13 only in Nantong, Jiangsu (C8); and H14 only in Kaiyang, Guizhou (C9). Chizhou (C7) contained the most haplotype types (H10, H11, and H12). Haplotype network analysis revealed H10 as the central, ancestral haplotype from which others were derived (Figure 1 [Figure 1: see original paper]).

2.3 Mismatch Analysis and Neutrality Tests

DNASP software was used for neutrality tests and mismatch analysis, yielding non-significant results. Conservative Tajima's D statistic was 0.67883 ($P > 0.10$); Fu and Li' s D* was 1.38007 ($0.10 > P > 0.05$); Fu and Li' s F* was 1.34421 ($P > 0.10$); and Fu' s Fs was -1.304. Positive Tajima' s D values suggest possible bottleneck effects. The mismatch distribution showed a bimodal pattern with discordance between expected and observed distributions, violating population expansion models. Neutrality tests detected no expansion signals, indicating a stable population history without large-scale expansion events (Figure 3 [Figure

3: see original paper]).

2.4 Genetic Diversity and Population Genetic Structure

PERMUT analysis revealed total genetic diversity (HT) of 0.882, average within-population diversity (HS) of 0.134, and differentiation coefficients GST of 0.855 and NST of 0.913. The pattern of NST $>$ GST ($0.01 < P < 0.05$) indicated significant phylogeographic structure, with closely related haplotypes co-occurring within the same populations.

AMOVA results showed that genetic variation occurred primarily among populations, with 89.27% of total variation attributed to among-population differences and only 10.73% from within-population variation (Table 4). The fixation index FST was 0.909 ($P < 0.001$), with 1,000 permutations confirming significance (FST > 0.25), demonstrating substantial genetic differentiation and isolation among *P. ternata* populations. Assuming drift-migration equilibrium, the estimated average gene flow (Nm) among populations was 0.02, indicating extremely low inter-population gene flow.

3.1 Genetic Diversity

Population genetic diversity is closely related to environmental conditions, with numerous factors directly or indirectly influencing genetic variation (Jiang, 2017). Generally, widespread species exhibit higher genetic diversity than narrowly distributed species (Hamrick, 1992). *P. ternata* has a broad distribution range with distinct genetic and morphological variations across different geographic environments. During field sampling, we observed different ecotypes varying in plant size, leaf shape, and spathe characteristics, consistent with our experimental data. Analysis of three cpDNA fragments from 212 individuals across 19 natural populations revealed total haplotype diversity (Hd) of 0.882 and nucleotide diversity (π) of 1.23×10^{-3}, exceeding the average cpDNA genetic variation of 0.67 reported for 170 species by Petit et al. (2005). Zhang (2021) reported average Nei' s gene diversity index (h) of 1.03 in 17 *P. ternata* populations using SSR markers, while Pan et al. (2021) obtained haplotype diversity (Hd) of 0.8596 in 20 populations using ITS sequences. Our results align with these studies, all indicating high genetic diversity at the species level. This high diversity may result from natural selection and species distribution range. Complex topography and diverse climates across different distribution areas create geographic isolation, leading to substantial differentiation in morphology, physiology, genetics, and ecological habits (Wang et al., 2011; Pan et al., 2021). As a widespread species, *P. ternata* likely harbors different genetic and morphological variations across environments. Additionally, gene mutation may contribute significantly, as *P. ternata* possesses both sexual and asexual reproduction systems with dominant vegetative propagation due to highly sterile male gametes (Wang et al., 2000). In plants with difficult sexual reproduction, gene mutation becomes a primary source of genetic variation (Wang et al., 2011), potentially contributing to high inter-population genetic diversity. Furthermore, *P. ternata* possesses a complex gene pool that may have accumulated rich chloroplast genetic variation through long evolutionary history and generational turnover, providing abundant material for mutation.

3.2 Geographic Distribution Pattern and Population Historical Dynamics

Plant geographic distribution patterns and genetic structure are influenced by geological and climatic history, habitat heterogeneity, and seed-mediated gene flow (Liu et al., 2021). This study revealed significant phylogeographic structure ($NST = 0.913 > GST = 0.855$, $0.01 < P < 0.05$) and substantial genetic differentiation ($FST = 0.909$, $P < 0.001$), indicating infrequent gene flow and significant geographic isolation or environmental heterogeneity. The estimated gene flow ($Nm = 0.02$) based on FST values suggests insufficient gene flow to counteract genetic drift-induced population differentiation when $Nm < 1$ (Slatkin & Montgomery, 1985). Therefore, we infer that geographic isolation (or environmental heterogeneity) and genetic drift are primary factors driving inter-population differentiation. Low gene flow may be attributed to: (1) Reproductive modes including seed, tuber, and bulbil propagation (Zhang et al., 2016). Bulbils are particularly important for reproduction, with their size and number closely related to tuber yield (Zhang et al., 2013). Bulbils, daughter tubers, and fruits remain near maternal plants, making *P. ternata* a poor colonizer (Gu & Guo, 1990) and resulting in low inter-population gene flow. (2) Habitat fragmentation due to geographic isolation and environmental factors, disrupting or weakening gene exchange among populations.

Non-significant Tajima’ s D and Fu’ s Fs values suggest the *P. ternata* population conforms to neutral evolution models. The bimodal mismatch distribution, with discordance between expected and observed values, indicates no recent large-scale expansion, contrasting with Pan et al. (2021) who suggested expansion events. This discrepancy may stem from cpDNA’ s maternal inheritance, where gene flow occurs primarily through seed dispersal (Hu & Li, 2002), whereas nuclear genes have biparental inheritance with both seed and pollen flow contributions. Limited seed dispersal compared to pollen may yield different results.

Haplotype geographic distribution shows multiple relatively isolated distribution zones, each with private and dominant haplotypes. Generally, haplotypes located centrally in network structures, with high frequency and wide distribution, are considered ancestral (Freeland et al., 2012). Haplotype H10, with high frequency, wide distribution, and central network position, is inferred as the ancestral haplotype. Chizhou, Anhui (C7) not only showed high haplotype diversity but also contained the most haplotype types, supporting the conclusion that *P. ternata* originated in eastern China and spread along the Yangtze River (Li, 1996). Fourteen of 19 populations contained only one haplotype, likely related to predominant vegetative reproduction, where a single genotype (clone) may dominate a population.

Glacial refugia refer to areas where species survived during ice ages, particularly the Last Glacial Maximum, when climate and topography changed dramatically, forcing large-scale geographic shifts (Haffer, 1969). According to refugia theory, regions with high genetic diversity, ancient haplotypes, and numerous private haplotypes are potential glacial refugia (Favre et al., 2010). Southwestern populations (Zhaotong, Yunnan (C6); Guiyang, Guizhou (C14); Mianyang, Sichuan (C17)) and eastern populations (Chizhou, Anhui (C7); Yiwu, Zhejiang (C13)) all exhibited high genetic diversity with ancient and private haplotypes. Therefore, at least two glacial refugia existed for *P. ternata* during the Quaternary ice ages, likely in central-eastern and southwestern China, consistent with our research group' s findings on *Allium macrostemon* (Mo et al., 2019; Shi et al., 2021), possibly due to similar distribution and ecological characteristics.

This study investigated genetic diversity, phylogeographic patterns, and population historical dynamics of *P. ternata* across 15 provinces (Guizhou, Zhejiang, Gansu, Sichuan, etc.), spanning northern and southern China and representing eastern, central, northwestern, and southwestern distribution zones, thereby covering the entire Chinese geographic range. Although Japanese and Korean Peninsula materials were not included, our conclusions show similarities and consistency with our group' s previous studies on *Allium macrostemon* and *Bupleurum longiradiatum*, as well as earlier research on *Ginkgo biloba* (Fan, 2014) and *Liquidambar formosana* (Sun, 2017) across southwestern and central-eastern China, demonstrating that our sampling strategy was feasible and conclusions reliable.

3.3 Molecular Screening and Conservation Strategies for Elite Germplasm of Medicinal Plant *P. ternata*

Pinellia ternata tubers are used medicinally for drying dampness, resolving phlegm, reversing adverse flow of qi, stopping vomiting, and dispersing masses. It is one of the most commonly used traditional Chinese medicines, clinically applied for anti-tumor, anti-fertility, anti-spasmodic, lipid-regulating, expectorant, anti-swelling, and coronary heart disease treatments (Wang et al., 2012). In recent years, excessive use of chemical fertilizers and pesticides (e.g., herbicides) and unregulated harvesting have destroyed wild habitats, causing rapid depletion of wild resources. Meanwhile, domestic and international demand continues to grow, particularly with global warming increasing lung diseases and market demand. Therefore, breeding elite germplasm or new varieties has become an urgent priority. Additionally, continuous cropping obstacles cause severe diseases and pests, reduced product quality and yield, and rising prices, with few new varieties available (An et al., 2018). A few red-tuber varieties with high yield and resistance have been discovered but lack complete breeding methods (Zhang et al., 2021). Studies show *P. ternata* fears direct sunlight, with moderate shading promoting growth but excessive shading causing poor growth or death (Chang, 2022). High temperatures ($ $32°C) or low winter temperatures induce seedling fall or dormancy, affecting yield (Zhang et al., 2004). Therefore, breeding targets include tolerance to intense light and high temperature, low temperature and humidity, and continuous cropping, aiming to extract superior new germplasm. Selection involves comparing biological characteristics, resistance identification, yield analysis, and reproductive traits, followed by field performance evaluation in resource nurseries targeting growth vigor, leaf number per plant, disease resistance, bulbil position and capacity, tuber shape and productivity. Chang (2022) associated yield traits with cytological characteristics, finding that *P. ternata* germplasm with smaller genomes may favor bulbil formation and underground tuber proliferation. Our molecular study identified Zhaotong, Yunnan (C6); Guiyang, Guizhou (C14); Mianyang, Sichuan (C17); Chizhou, Anhui (C7); and Yiwu, Zhejiang (C13) as regions with high genetic diversity, representing distribution areas of wild elite germplasm.

Beyond breeding elite varieties, wild resources require conservation measures. In situ or ex situ conservation should be implemented in high-diversity regions. Given its wide distribution but small wild populations, ex situ conservation should be prioritized, such as establishing medicinal plant germplasm resource nurseries (banks) focusing on high-diversity populations. Populations with unique haplotypes, including Dandong, Liaoning (C1); Chizhou, Anhui (C7); Nantong, Jiangsu (C8); Kaiyang, Guizhou (C9); Qingdao, Shandong (C10); Yiwu, Zhejiang (C13); Guiyang, Guizhou (C14); Mianyang, Sichuan (C17); and Guyuan, Ningxia (C18), should receive priority protection. Additionally, individuals with special traits should be incorporated into conservation strategies.

References

AN Y, YANG D, LI X, et al., 2018. Study on the obstacle effect and physiological mechanism of *Pinellia ternata* continuous cropping[J]. J NW Agric, 27(7):1017-1022. [AN Yan, YANG Dan, LI Xin, et al., 2018. Study on the obstacle effect and physiological mechanism of continuous cropping of *Pinellia ternata*[J]. Acta Agriculturae Boreali-occidentalis Sinica, 27(7): 1017-1022.]

AVISE JC, ARNOLD J, BALL RM, et al., 1987. Intraspecific phylogeography; the mitochondrial DNA bridge between population genetics and systematics[J]. Ann Rev Ecol Syst, 18(1): 489-522.

AVISE JC, 1998. The history and purview of phylogeography: a personal reflection[J]. Mol Ecol, 7(4): 371-379.

AVISE JC, 2000. Phylogeography: The history and formation of species[M]. Cambridge, Massachusetts, London, England: Harvard University Press: 1-464.

BANDELT HJ, FORSTER P, ROHL A, 1999. Median-joining networks for inferring intraspecific phylogenies[J]. Mol Biol Evol, 16(1): 37-48.

BENNETT KD, PROVAN J, 2008. What do we mean by 'refugia' [J]. Quaternary Sci Rev, 27: 2449-2455.

CHANG Q, 2022. Genetic diversity analysis and excellent germplasm selection of *Pinellia ternata*[D]. Guiyang: Guizhou University. [CHANG Qin, 2022. Genetic diversity analysis and excellent germplasm selection of *Pinellia ternata*[D]. Guiyang: Guizhou University.]

CHEN LM, HE ZG, HAN RL, 2020. Advances in germplasm resources of *Pinellia ternata*[J]. Heilongjiang Agric Sci, (2): 131-135. [CHEN Liming, HE Zhigui, HAN Ruilian, 2020. Research progress on germplasm resources of *Pinellia ternata*[J]. Heilongjiang Agricultural Sciences, (2): 131-135.]

DARWIN CR, 1859. On the origin of species by means of natural selection, or the preservation of favored races in the struggle for life—By Charles Darwin, M.A., F.R.S., F.G.S., &c. London[J]. Ann Mag Nat Hist, 5(26): 132-143.

DU J, 2006. Comparative study on comprehensive characters of *Pinellia ternata* in different populations[D]. Beijing: Capital Normal University: 1-79. [DU Juan, 2006. Comparative study on comprehensive characters of *Pinellia ternata* in different populations[D]. Beijing: Capital Normal University: 1-79.]

EXOFFIER L, LISCHER HEL, 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows[J]. Mol Ecol Notes, 10(3): 564-567.

FAN XX, 2014. The research on the variety of genetic structure of *Ginkgo biloba* based on cpDNA haplotypes[J]. Nat Sci J Harbin Norm Univ, 30(4): 104-107. [FAN Xiaoxia, 2014. Research on genetic diversity pattern of *Ginkgo biloba* based on cpDNA haplotypes[J]. Natural Science Journal of Harbin Normal University, 30(4): 104-107.]

FAVRE A, YUAN YM, KUPFER P, et al., 2010. Phylogeny of subtribe Gentianinae (Gentianaceae): biogeographic inferences despite limitations in temporal calibration points[J]. Taxon, 59(6): 1701-1711.

FREELAND JR, KIRK H, PETERSEN SD, 2012. Phylogeography[M]. Mol Ecol. 2nd ed. New Jersey: Wiley-Blackwell: 225-269.

FU YX, 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection[J]. Genetics, 147(2): 915-925.

GU DX, GUO QS, 1990. A study on the biological characteristics of *Pinellia ternata* populations[J]. J Nanjing Agric Univ, 13(2): 11-16. [GU Dexing, GUO Qiaosheng, 1990. A study on the biological characteristics of *Pinellia ternata* populations[J]. Journal of Nanjing Agricultural University, 13(2): 11-16.]

HAFFER J, 1969. Speciation in Amazonian forest birds[J]. Science, 165(3889): 131-137.

HAMRICK JL, 1992. Factors influencing levels of genetic diversity in woody plant species[J]. New For, 6(1-4): 95-124.

HARRISON S, YU G, TAKAHARA H, et al., 2001. Palaeovegetation-Diversity of temperate plants in east Asia[J]. Nature, 413(6852): 129-130.

HEWITT GM, 1996. Some genetic consequences of ice ages, and their role in divergence and speciation[J]. Biol J Linn Soc, 58(3): 247-276.

HEWITT GM, 2004. Genetic consequences of climatic oscillations in the Quaternary[J]. Philos Trans R Soc B, 359(1442): 183-195.

HICKERSON MJ, CARSTENS BC, CAVENDER-BARES J, et al., 2010. Phylogeography' s past, present and future: 10 years after[J]. Mol Phylogenet Evol, 54: 291-301.

HONG X, CHEN XS, WANG JY, et al., 2021. Sequence characteristics and genetic diversity analysis of chloroplast gene trnH-psbA in taro germplasm[J]. J Zhejiang Agric Sci, 62(11): 2274-2276. [HONG Xia, CHEN Xiaoshang, WANG Jiaoyang, et al., 2021. Sequence characteristics and genetic diversity analysis of chloroplast gene trnH-psbA in taro germplasm[J]. Journal of Zhejiang Agricultural Sciences, 62(11): 2274-2276.]

HU XS, LI B, 2002. Seed and pollen flow and cline discordance among genes with different modes of inheritance[J]. Heredity, 88(3): 212-217.

HU YC, MA H, ZHU JZ, et al., 2021. Analysis of genetic diversity of ancient *Lycium barbarum* trees based on matK, psbA-trnH, rbcL-a, trnL-trnF sequences[J/OL]. Mol Plant Breed: 1-14. [HU Yongchao, MA Jie, ZHU Jinzhong, et al., 2021. Analysis of genetic diversity of ancient *Lycium barbarum* trees based on matK, psbA-trnH, rbcL-a, trnL-trnF sequences[J/OL]. Molecular Plant Breeding: 1-14.]

JIANG FJ, 2017. Phylogeography of *Lilium pumilum* Redouté in southeast of Qinghai-Tibetan Plateau[D]. Xining: Qinghai University: 1-74. [JIANG Fujuan, 2017. Phylogeography of *Lilium pumilum* Redouté in southeast of Qinghai-Tibetan Plateau[D]. Xining: Qinghai University: 1-74.]

LI H, 1996. The ecological phytogeography and origin of the family Araceae[J]. Acta Bot Yunnan, 18(1): 14-42. [LI Heng, 1996. The ecological phytogeography and origin of the family Araceae[J]. Acta Botanica Yunnanica, 18(1): 14-42.]

LI YX, LIU XD, ZHU ZJ, 2004. Progress on the research of pharmacology of *Pinellia ternata*[J]. J Liaoning Univ Tradit Chin Med, 6(6): 459-460. [LI Yuxian, LIU Xiaodong, ZHU Zhaojing, 2004. Progress on the research of pharmacology of *Pinellia ternata*[J]. Journal of Liaoning University of Traditional Chinese Medicine, 6(6): 459-460.]

LI Z, XUAN J, ZHAO ZH, et al., 2021. Research Progress on chemical constituents and pharmacological effects of Banxia (*Pinelliae Rhizoma*)[J]. J Liaoning Univ Tradit Chin Med, 23(11): 154-158. [LI Zhe, XUAN Jing, ZHAO Zhenhua, et al., 2021. Research progress on chemical constituents and pharmacological effects of Banxia (*Pinelliae Rhizoma*)[J]. Journal of Liaoning University of Traditional Chinese Medicine, 23(11): 154-158.]

LIBRADO P, ROZAS J, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data[J]. Bioinformatics, 25(11): 1451-1452.

LIU JQ, SUN YS, GE XJ, et al., 2012. Phylogeographic studies of plants in China: advances in the past and directions in the future[J]. J Syst Evol, 50(4): 267-275.

LIU JQ, FU MJ, WU HY, et al., 2021. Phylogeography of the Loess Plateau plant *Prinsepia uniflora* inferred from the chloroplast DNA[J]. Guihaia, 41(3): 396-403. [LIU Jiaqi, FU Mengjiao, WU Haiyang, et al., 2021. Phylogeography of the Loess Plateau plant *Prinsepia uniflora* inferred from the chloroplast DNA[J]. Guihaia, 41(3): 396-403.]

MENG HH, GAO XY, HUANG JF, et al., 2015. Plant phylogeography in arid Northwest China: Retrospectives and perspectives[J]. J Syst Evol, 53(1): 33-46.

MO ZM, YANG CD, LI YS, et al., 2022. Analysis of genetic diversity and geographical distribution pattern of *Spiranthes sinensis*[J]. Mol Plant Breed, 20(3): 1037-1044. [MO Zhongmei, YANG Chuandong, LI Yinshuang, et al., 2022. Analysis of genetic diversity and geographical distribution pattern of *Spiranthes sinensis*[J]. Molecular Plant Breeding, 20(3): 1037-1044.]

MO ZM, CHENG Y, SHI T, et al., 2019. Genetic relationship and geographical distribution pattern of different *Allium macrostemon* populations in southwest China based on ITS sequences[J]. Acta Bot Boreal-Occident Sin, 39(9): 1573-1580. [MO Zhongmei, CHENG Yu, SHI Tian, et al., 2019. Genetic relationship and geographical distribution pattern of different *Allium macrostemon* populations in southwest China based on ITS sequences[J]. Acta Botanica Boreali-Occidentalia Sinica, 39(9): 1573-1580.]

PAN F, MO ZM, SHI T, et al., 2021. Genetic diversity and genetic structure analysis of *Pinellia ternata* (Thunb.) Breit populations[J]. Mol Plant Breed, 19(24): 8347-8352. [PAN Feng, MO Zhongmei, SHI Tian, et al., 2021. Genetic diversity and genetic structure analysis of *Pinellia ternata* (Thunb.) Breit populations[J]. Molecular Plant Breeding, 19(24): 8347-8352.]

PETIT RJ, DUMINIL J, FINESCHI S, et al., 2005. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations[J]. Mol Ecol, 14(3): 689-701.

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[J]. Mol Phylogenet Evol, 59(1): 225–244.

SHAFER A, CULLINGHAM CI, CÔTÉ SD, et al., 2010. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America[J]. Mol Ecol, 19(21): 4589–4621.

SHI T, MO ZM, WU M, et al., 2021. Phylogeography of medicinal and edible homologous plant *Allium macrostemon*[J]. Bull Bot Res, 42(4): 574-583. [SHI Tian, MO Zhongmei, WU Min, et al., 2021. Phylogeography of medicinal and edible homologous plant *Allium macrostemon*[J]. Bulletin of Botanical Research, 42(4): 574-583.]

SLATKIN M, 1985. Gene Flow in Natural Populations[J]. Ann Rev Ecol Syst, 16: 393-430.

SOLTIS DE, MORRIS AB, MCLACHLAN JS, et al., 2006. Comparative phylogeography of unglaciated eastern North America[J]. Mol Ecol, 15(14): 4261–4293.

SUDHIR K, GLEN S, KOICHIRO T, 2016. MEGA7: Molecular evolutionary genetics analysis Version 7.0 for bigger datasets[J]. Mol Biol Evol, 33(7): 1870-1874.

SUN RX, 2017. Genetic diversity and geography of *Liquidambar formosana* Hance in China[D]. Beijing: Chinese Academy of Forestry Sciences: 1-132. [SUN Rongxi, 2017. Genetic diversity and geography of *Liquidambar formosana* Hance in China[D]. Beijing: Chinese Academy of Forestry Sciences: 1-132.]

TAJIMA F, 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism[J]. Genetics, 123(3): 585-595.

TZEDAKIS PC, LAWSON IT, FROGLEY MR, et al., 2002. Buffered tree population changes in a quaternary refugium: evolutionary implications[J]. Science, 297(5589): 2044-2047.

WANG AH, JI XB, WENG XP, 2012. Genetic diversity of 16 wild *Pinellia ternata* germplasm[J]. Guizhou Agric Sci, 40(1): 15-19. [WANG Aihua, JI Xiangbiao, WEN Xiaopeng, 2012. Genetic diversity analysis of 16 wild *Pinellia ternata* germplasm[J]. Guizhou Agricultural Sciences, 40(1): 15-19.]

WANG HW, GE S, 2010. Phylogeography of the endangered *Cathaya argyrophylla* (Pinaceae) inferred from sequence variation of mitochondrial and nuclear DNA[J]. Mol Ecol, 15(13): 4109-4122.

WANG YH, COMES HP, CAO YN, et al., 2017. Quaternary climate change drives allo-peripatric speciation and refugial divergence in the *Dysosma versipellis-pleiantha* complex from different forest types in China[J]. Sci Rep-UK, 7(1): 40261.

WANG ZX, PENG ZS, HE YK, 2000. Genetic analysis of male gamete abortion of *Pinellia ternata*[J]. J Crops, 26(1): 83-86. [WANG Zuxiu, PENG Zhengsong, HE Yikun, 2000. Genetic analysis of male gamete abortion of *Pinellia ternata*[J]. Acta Agronomica Sinica, 26(1): 83-86.]

WEI G, CHUAN C, CHRISTOPH D, et al., 2008. Phylogeography of a living fossil: Pleistocene glaciations forced *Ginkgo biloba* L. (Ginkgoaceae) into two refuge areas in China with limited subsequent postglacial expansion[J]. Mol Phylogenet Evol, 48(3): 1094-1105.

YE JW, BAI WN, BAO L, et al., 2017. Sharp genetic discontinuity in the aridity-sensitive species *Lindera obtusiloba* (Lauraceae): Solid evidence supporting the tertiary floral subdivision in East Asia[J]. J Biogeogr, 44(9): 2082–2095.

ZHANG D, GAO F, JAKOVLI I, et al., 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies[J]. Mol Ecol Resour, 20(1): 348-355.

ZHANG JJ, HUANG Y, ZHANG DQ, et al, 2021. Phylogeography of *Paris polyphylla* var. *yunnanensis* based on chloroplast gene trnL-trnF sequences[J]. Chin J Chin Mat Med, 46(5): 1094-1101. [ZHAO Jingjing, HUANG Yu, ZHANG Dequan, et al., 2021. Phylogeography of *Paris polyphylla* var. *yunnanensis* based on chloroplast gene trnL-trnF sequences[J]. Chinese Journal of Chinese Materia Medica, 46(5): 1094–1101.]

ZHANG JY, 2007. Study on genetic diversity of *Pinellia ternata* by molecular markers and quality assessment[D]. Nanjing: Nanjing Agricultural University: 1-140. [ZHANG Junyi, 2007. Study on genetic diversity of *Pinellia ternata* by molecular markers and quality assessment[D]. Nanjing: Nanjing Agricultural University: 1-140.]

ZHANG JY, GUO QS, WU LW, et al., 2006. Ribosomal DNA ITS sequence analysis of *Pinellia ternata* from different geographical regions in China[J]. Chin J Chin Mat Med, 31(21): 1768-1772. [ZHANG Junyi, GUO Qiaosheng, WU Liwei, et al., 2006. Ribosomal DNA ITS sequence analysis of *Pinellia ternata* from different geographical regions in China[J]. Chinese Journal of Chinese Materia Medica, 31(21): 1768-1772.]

ZHANG J, LI XD, ZONG QB, et al., 2021. Development of SSR molecular markers and genetic diversity analysis in *Pinellia ternata* (Thunb.) Breit[J]. J Huazhong Agric Univ, 40(6): 19-26. [ZHANG Jing, LI Xiaodong, ZONG Qingbo, et al., 2021. Development of SSR molecular markers and genetic diversity analysis in *Pinellia ternata* (Thunb.) Breit[J]. Journal of Huazhong Agricultural University, 40(6): 19-26.]

ZHANG XY, YANG MQ, XU Y, et al., 2019. Study on the genetic diversity and genetic structure of *Cardiocrinum giganteum* based on rpl16 sequences analysis[J]. J Plant Genet Resour, 20(1): 199–206. [ZHANG Xiaoyun, YANG Miaoqin, XU Ying, et al., 2019. Study on the genetic diversity and genetic structure of *Cardiocrinum giganteum* based on rpl16 sequences analysis[J]. Journal of Plant Genetic Resources, 20(1): 199–206.]

ZHANG YF, 2022. Application of chloroplast genome in botany research[J]. Biol Teach, 47(3): 83-85. [ZHAO Yufen, 2022. Application of chloroplast genome in botany research[J]. Biology Teaching, 47(3): 83-85.]

ZHANG M, ZHONG GY, MA KS, et al., 2004. Experimental observation on the causes of *Pinellia ternata* seedling fall[J]. Chin J Chin Mat Med, 29(3): 85-86. [ZHANG Ming, ZHONG Guoyue, MA Kaisen, et al., 2004. Experimental observation on the causes of *Pinellia ternata* seedling fall[J]. Chinese Journal of Chinese Materia Medica, 29(3): 85-86.]

ZHAO YM, LI XL, 2016. An application of matK and rbcL in *Pinellia*[J]. J Shangluo Univ, 30(6): 67-70. [ZHAO Yuemei, LI Xiaoling, 2016. An application of matK and rbcL in *Pinellia*[J]. Journal of Shangluo University, 30(6): 67-70.]

ZHENG DS, ZHANG JY, GUO QS, 2013. cpDNA non-coding sequence analysis of *Pinellia ternata* and its related species[J]. Chin Tradit Herb Drugs, 44(07): 881-886. [ZHENG Danshu, ZHANG Junyi, GUO Qiaosheng, 2013. cpDNA non-coding sequence analysis of *Pinellia ternata* and its related species[J]. Chinese Traditional and Herbal Drugs, 44(07): 881-886.]

Note: Figure translations are in progress. See original paper for figures.