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

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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) from 212 individuals across 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 revealed: (1) The total haplotype diversity (Hd) of P. 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) demonstrated that genetic variation primarily occurred among populations, with significant genetic differentiation (FST = 0.909, $P < 0.001$) and low within-population genetic diversity (HS = 0.134). The among-population genetic differentiation coefficient NST = 0.913 > GST = 0.855 ($0.01 < P < 0.05$) suggested 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* were non-significant positive values, while Fu's Fs was a non-significant negative value, and the mismatch distribution curve exhibited a bimodal pattern, indicating that P. ternata populations have not experienced overall expansion events. (4) The geographic distribution of haplotypes revealed higher haplotype diversity in southwestern and central-eastern regions, with the presence of endemic haplotypes, leading to the inference that glacial refugia existed in these two regions during the Quaternary glacial periods. In summary, through the analysis of three chloroplast genes in P. ternata from different regions, this study elucidates its genetic diversity, genetic structure, and geographical distribution patterns, and provides scientific recommendations and conservation strategies for the molecular screening and protection of superior germplasm resources of P. ternata.

Full Text

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

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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 molecular 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*, aiming to investigate its genetic diversity, genetic structure, geographic distribution patterns and their underlying causes, and to explore its population historical dynamics. The results revealed: (1) Total haplotype diversity (Hd) of *P. ternata* was 0.882, with 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 was NST = 0.913 > GST = 0.855 ($0.01 < P < 0.05$), demonstrating a clear 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, while Fu's Fs was a non-significant negative value, and mismatch analysis revealed a bimodal curve, indicating that *P. ternata* populations have not experienced expansion events. (4) Haplotype geographic distribution showed that southwestern and central-eastern regions exhibited high haplotype diversity with endemic haplotypes, suggesting the existence of glacial refugia in these two regions during the Quaternary glaciation. In summary, analysis of three chloroplast genes across different regions of *P. ternata* clarifies its genetic diversity, genetic structure, and geographic distribution patterns, and provides scientific recommendations and conservation strategies for molecular screening and protection of superior germplasm resources.

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 in high-latitude regions to retreat southward, forming "glacial refugia" (Hewitt, 2004). Conversely, during interglacial or postglacial periods when climate warmed and glaciers retreated, plants from refugia migrated back to high-latitude regions (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 large-scale 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 glacial refugia experienced range expansions; (3) The uplift of the Qinghai-Tibet Plateau causing mountain and river isolation, monsoon-induced dry-wet alternation, aridity, and Quaternary glacial-interglacial cycles constitute the main 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 plants.

Plant chloroplast DNA (cpDNA) is a circular double-stranded DNA molecule that, compared to nuclear genomic DNA, features small molecular weight, multiple copies, and simple structure, with rare recombination among different cpDNA fragments. Consequently, cpDNA exhibits relatively high genetic differentiation and low 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, an increasing number of studies have employed multiple chloroplast genes to investigate plant genetic diversity, genetic structure, phylogeographic distribution patterns, and population historical dynamics (Zhao, 2022).

Pinellia ternata (Araceae) is a perennial herbaceous plant, commonly known as Shoutian, Diwen, or by folk names such as Mayutou, Tianluoxing, and Wuxincai. It is a clinically important traditional Chinese medicine that grows in moist, warm, shaded, and loose sandy soils below 2,500 m altitude, and is endemic to East Asia. Wild *P. ternata* is distributed across all provinces in China except Inner Mongolia, Qinghai, Xinjiang, and Tibet (Li et al., 2004; Zhang, 2007). Due to geographic distance, genetic and phenotypic differentiation have occurred

over time, affecting its genetic diversity and genetic structure. Meanwhile, extensive commercial exploitation and lack of conservation measures have led to a sharp decline in wild resources, necessitating collection and genetic background analysis of wild germplasm. As the utilization scope of *P. ternata* continues to expand, related research has become increasingly abundant, though primarily focusing on chemical composition, toxicity, and pharmacological effects (Li et al., 2021).

Few scholars have studied the phylogeny and evolutionary relationships of *P. ternata*. For instance, Zhao & Li (2016) used matK+rbcL combined sequences to analyze the phylogenetic relationships of *Pinellia* species, demonstrating that matK+rbcL combined sequences could serve as DNA barcodes for species identification. Zhang (2007) used ITS sequences to analyze sequence differences among *P. ternata* from different regions in China and their correlation with geographic distribution and external morphology, showing that rDNA variation was related to geographic distribution. Pan et al. (2021) used ITS sequences to reveal the population genetic structure and genetic diversity of *P. ternata*. Zhang et al. (2021) used screened SSR primers to analyze genetic diversity among different populations. Zheng et al. (2013) used chloroplast non-coding regions (psbK-psbI and atpF-atpH) to analyze sequences of *P. ternata* and related species, obtaining relatively rich variable sites. However, comprehensive studies on genetic diversity, genetic structure, phylogeographic distribution patterns, and population historical dynamics of *P. ternata* based on multiple chloroplast genes remain lacking. As a herbaceous, widely distributed species with strong ecological adaptability spanning different climate zones and multiple biodiversity hotspots, *P. ternata* serves as an ideal material for investigating genetic structure, phylogeographic distribution patterns, and population historical dynamics of herbaceous and widespread species. This study employs phylogeographic methods based on three chloroplast genes (psbK-psbI, atpF-atpH, and trnL-F) to analyze 212 wild individuals from 19 natural populations across 15 provinces in eastern, central, northwestern, and southwestern China, aiming to explore the genetic diversity, genetic structure, phylogeographic distribution patterns, and population historical dynamics of this medicinal plant. The objectives are to reveal genetic variation among and within different populations, elucidate haplotype geographic distribution patterns, infer glacial refugia during the Quaternary glaciation, and propose scientific recommendations and conservation strategies for molecular screening and protection of superior germplasm. This study provides important references for resource conservation, utilization, and molecular-assisted breeding of *P. ternata*, while also offering theoretical foundations for further exploring the evolution of Chinese flora and the formation of species diversity.

Materials and Methods

1.1 Plant 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 information measured using Global Positioning System (Table 1). Fresh leaves of *P. ternata* were immediately dried with silica gel in the field for total DNA extraction. Voucher specimens were deposited in the Herbarium of the College of Life Sciences, Guizhou University.

1.2 DNA Extraction, Amplification, and 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 sequences of *P. ternata* and related reports (Zheng et al., 2013), with three chloroplast gene fragments (psbK-psbI, atpF-atpH, and trnL-F) screened and synthesized by Sangon Biotech (Shanghai) Co., Ltd. Primer sequences and PCR amplification protocols are shown in Table 2 . The PCR reaction system was 25 L, containing 12.5 L of 2× Taq PCR MasterMix, 8.5 L ddH_2O, 1 L forward primer, 1 L reverse primer, and 2 L DNA template. Total DNA and PCR products were detected using 1% agarose gel electrophoresis. After electrophoresis, gels were placed in a gel imaging system for observation. Products meeting quality standards were sent to Sangon Biotech (Shanghai) Co., Ltd. for purification and sequencing.

1.3 Data Analysis

MEGA7.0 software (Sudhir et al., 2016) was used for multiple sequence alignment, error base correction, sequence sorting, manual correction, and removal of primer regions at both ends. PhyloSuite software (Zhang et al., 2020) was employed for sequence assembly. DNASP6.0 software was used to count haplotype numbers and calculate haplotype diversity (Hd), nucleotide diversity (π), Tajima's D value, Fu and Li's F* value, Fu & Li's D value, and Fu's Fs value for each population, as well as to conduct mismatch analysis 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 genetic differentiation coefficients (GST and NST), with Network used to construct haplotype network diagrams (Bandelt et al., 1999). Arlequin software (Lu, 2018) was applied for AMOVA analysis to calculate population genetic structure and detect genetic variation within and among populations, as well as to calculate genetic differentiation coefficient (FST) and gene flow (Nm) to further reveal population differentiation levels. ArcGIS 10.2 software was used to map geographic distribution of population haplotypes.

Results

2.1 Sequence Variation and Haplotype Diversity

The combined psbK-psbI+atpF-atpH+trnL-F sequences were successfully sequenced for 212 individuals, with an aligned length of 1,947 bp after correction, containing 11 variable sites and a G + C content of 31.80%. These sequences were submitted to GenBank with 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) possessed 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} for *P. ternata*. Zhaotong City, Yunnan (C6) and Mianyang City, Sichuan (C17) exhibited the highest haplotype diversity (0.5333), followed by Chizhou City, Anhui (C7) and Yiwu City, Zhejiang (C13). Overall, populations in southwestern and eastern China showed higher genetic diversity levels.

2.2 Haplotype Distribution

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

2.3 Mismatch Analysis and Neutrality Tests

DNASP software was used for neutrality tests and mismatch analysis of the sequences, with neutrality tests showing non-significant results. The 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 that *P. ternata* populations may have experienced bottleneck effects. The mismatch distribution showed a bimodal pattern, with expected values contradicting observed values, violating the population expansion model. Neutrality tests also detected no signals of population expansion, indicating a stable population history without overall 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 genetic diversity (HS) of 0.134, and inter-population genetic differentiation coefficients GST of 0.855 and NST of 0.913. The genetic differentiation coefficient NST > GST ($0.01 < P < 0.05$) indicated a distinct molecular phylogeographic structure, where closely related different haplotypes co-occurred within the same population.

AMOVA results showed that genetic variation occurred primarily among populations, with 89.27% of total variation among the 19 *P. ternata* populations and only 10.73% within populations (Table 4). The genetic differentiation coefficient FST was 0.909 ($P < 0.001$), with significance testing over 1,000 replications showing FST > 0.25, indicating that genetic differentiation among *P. ternata* populations had reached a significant level with obvious isolation. Assuming drift-migration equilibrium for fragment variation, the average gene flow value (Nm) among populations at the species level was estimated as 0.02 based on FST values, indicating low gene flow among *P. ternata* populations.

Discussion

3.1 Genetic Diversity

Population genetic diversity is closely related to its living environment, with many environmental factors directly or indirectly affecting genetic diversity (Jiang, 2017). Generally, widespread species exhibit higher genetic diversity than narrowly distributed species (Hamrick, 1992). *Pinellia ternata* has a broad distribution range with different genetic and morphological variations across geographic environments. During field sampling, we observed different ecotypes of *P. ternata* showing variation in plant size, leaf shape, and spathe, consistent with our experimental data. Analysis of three cpDNA fragments from 212 individuals across 19 natural populations revealed total haplotype diversity of $Hd = 0.882$ and nucleotide diversity of $\pi = 1.23 \times 10^{-3}$, higher than the average chloroplast genetic variation of 0.67 reported for 170 species by Petit et

al. (2005). Zhang et al. (2021) used SSR molecular markers to study 17 *P. ternata* populations and found an average Nei's gene diversity index (h) of 1.03, while Pan et al. (2021) used ITS sequences to analyze 20 *P. ternata* populations and obtained haplotype diversity (Hd) of 0.8596. Our results are consistent with these studies, all indicating high genetic diversity at the species level in *P. ternata*. This outcome may be attributed to two factors. First, natural environmental selection: research shows that species distribution range is closely related to genetic diversity. Complex topography and diverse climate changes in different distribution areas or habitats lead to certain geographic isolation, causing significant differentiation in morphology, physiology, genetics, and ecological habits among populations (Wang et al., 2011; Pan et al., 2021). As a widespread species, *P. ternata* likely harbors different genetic and morphological variations across different geographic environments. Our field observations of different ecotypes varying in plant size, leaf shape, and spathe support this inference. Second, gene mutation may play a role: research indicates that *P. ternata* possesses both sexual and asexual reproductive systems, with asexual reproduction being dominant due to highly sterile male gametes (Wang et al., 2000). In plants with difficult sexual reproduction, gene mutation becomes a major factor causing genetic variation (Wang et al., 2011), potentially contributing to high genetic diversity among populations. Additionally, *P. ternata* has a complex gene pool that may have accumulated rich chloroplast genetic variation during its long evolutionary history and generational alternation, providing abundant material basis for gene mutation.

3.2 Geographic Distribution Pattern and Population Historical Dynamics

Plant population geographic distribution patterns and genetic structure are influenced by geological and historical climate changes, habitat heterogeneity, and seed-mediated gene exchange (Liu et al., 2021). This study found that *P. ternata* exhibits a clear phylogeographic structure ($NST = 0.913 > GST = 0.855$, $0.01 < P < 0.05$) with significant genetic differentiation among populations ($FST = 0.909$, $P < 0.001$), indicating infrequent gene exchange among populations and significant geographic isolation or environmental heterogeneity. Based on FST values, the estimated average gene flow (Nm) among populations at the species level was 0.02. When $Nm < 1$, gene flow is insufficient to counteract population differentiation caused by genetic drift within populations (Slatkin & Montgomery, 1985). Therefore, we infer that geographic isolation (or environmental heterogeneity) and genetic drift are the main factors causing genetic differentiation among *P. ternata* populations. Low gene flow among populations can be attributed to two primary reasons: (1) Reproductive modes of *P. ternata* (seed propagation, tuber propagation, and bulbil propagation) (Zhang et al., 2016). Bulbils are particularly important for reproduction, with their size and quantity closely related to tuber yield (Zhang et al., 2013). Bulbils, daughter tubers, and fruits all remain near maternal plants, lacking effective dispersal mechanisms and making *P. ternata* a poor colonizer (Gu & Guo, 1990), result-

ing in low gene flow among populations and high genetic differentiation. (2) Geographic isolation and environmental factors have caused habitat fragmentation in *P. ternata*, interrupting or weakening gene exchange among different populations or regional groups. Neutrality test results showed non-significant Tajima's D and Fu's Fs values, indicating that *P. ternata* populations conform to neutral evolution models. The bimodal mismatch distribution, with contradictory expected and observed values, suggests that *P. ternata* has not experienced recent large-scale expansion, contrary to Pan et al. (2021) who suggested expansion occurred. This discrepancy may be related to the maternal inheritance of chloroplast DNA, where gene flow occurs primarily through seed dispersal (Hu & Li, 2002), whereas nuclear genes are biparentally inherited with both seed and pollen flow contributing to gene flow. Compared to pollen dispersal, seed dispersal is more limited, leading to inconsistent results.

Haplotype geographic distribution revealed multiple relatively isolated distribution areas, each containing its own endemic and major haplotypes. Research indicates that haplotypes located in central positions of network structures, with high frequency and wide geographic distribution, are generally more ancient (Freeland et al., 2012). In this study, haplotype H10 showed high frequency, wide distribution, and central network position, suggesting it as the most ancient haplotype. Chizhou City, Anhui (C7) not only had high haplotype diversity but also the greatest number of haplotype types, consistent with the conclusion that *P. ternata* originated in eastern China and diffused along the Yangtze River to surrounding areas (Li, 1996). Among the 19 populations, 14 contained only one haplotype, likely related to within-population variation levels and reproductive modes. For *P. ternata*, which primarily reproduces vegetatively, a population likely contains only one genotype, i.e., a single clone. Glacial refugia typically refer to areas where species survived during glacial periods, particularly the Last Glacial Maximum, when climate and topography underwent dramatic changes causing large-scale geographic shifts in biological species (Haffer, 1969). According to glacial refugia theory, areas with high genetic diversity, ancient haplotypes, and numerous endemic haplotypes may represent potential glacial refugia for a species during ice ages (Favre et al., 2010). Since Zhaotong City, Yunnan (C6), Guiyang City, Guizhou (C14), and Mianyang City, Sichuan (C17) in southwestern China, as well as Chizhou City, Anhui (C7) and Yiwu City, Zhejiang (C13) in eastern China all showed high genetic diversity and contained ancient and endemic haplotypes, *P. ternata* likely had at least two glacial refugia during the Quaternary glaciation. We hypothesize that glacial refugia for *P. ternata* existed in central-eastern and southwestern China during the Quaternary ice age, consistent with our research group's findings on *Allium macrostemon* (Mo et al., 2019; Shi et al., 2021), likely related to their similar distribution and ecological habits.

This study analyzed genetic diversity, phylogeographic patterns, and population historical dynamics of *P. ternata*, with sampling covering 15 provinces including Guizhou, Zhejiang, Gansu, and Sichuan, spanning northern and southern China and representing natural geographic distributions in eastern, central, northwest-

ern, and southwestern regions, thus presenting the complete geographic distribution of this plant in China. Although we did not collect materials from Japan and the Korean Peninsula, our conclusions show similarities and consistency with our research group's previous studies on phylogeography and genetic diversity of herbaceous plants with similar geographic distributions such as *Allium macrostemon* and *Bupleurum longiradiatum*, as well as previous research on *Ginkgo biloba* (Fan, 2014) and *Liquidambar formosana* (Sun, 2017) that span southwestern and central-eastern China. Therefore, our sampling site selection was feasible and our conclusions are reliable.

3.3 Molecular Screening and Conservation Strategies for Superior Germplasm of Medicinal Plant *Pinellia ternata*

The tubers of *P. ternata* are used medicinally for drying dampness and resolving phlegm, descending counterflow and stopping vomiting, and clearing glomus and dispersing masses, making it one of the most commonly used traditional Chinese medicines with clinical applications in anti-tumor, anti-fertility, antispasmodic, lipid-regulating, expectorant, anti-swelling, and coronary heart disease treatments (Wang et al., 2012). In recent years, excessive use of chemical fertilizers and pesticides (such as herbicides) in farmland, disorderly resource exploitation, and continuous reclamation of wasteland have destroyed the wild growth environment of *P. ternata*, causing its wild resources to become increasingly depleted. Additionally, domestic and international demand for *P. ternata* is growing daily, especially with global warming increasing lung diseases and market demand. Therefore, breeding superior germplasm or cultivating new varieties has become an urgent practical need for *P. ternata* production development. Furthermore, continuous cropping obstacles lead to serious diseases and pests, reduced product quality, decreased yield, and increased prices, with few (or no) new varieties available (An et al., 2018). Currently, a very few *P. ternata* varieties with red tubers have been found to possess high yield and resistance characteristics, yet no complete breeding method exists (Zhang et al., 2021). Research indicates that *P. ternata* fears direct sunlight, with moderate shading promoting growth and development while excessive shading causes poor growth or even death (Chang, 2022). High temperatures ($ $32°C) or excessively low winter temperatures both cause seedling fall or dormancy in *P. ternata*, affecting yield (Zhang et al., 2004). Therefore, breeding directions focus on tolerance to strong light and high temperature, low temperature and humidity, and continuous cropping, aiming to extract superior new germplasm from *P. ternata* resources. Selected breeding samples were screened through comparison of biological characteristics, resistance identification, yield analysis, and reproductive trait studies to identify superior lines, which were then evaluated based on field performance in resource gardens targeting main indicators including growth vigor, leaf number per plant, disease resistance, bulbil position and production capacity, tuber shape and productivity. Chang (2022) associated yield traits with cytological characteristics in *P. ternata*, finding that germplasm with smaller genomes seemed more conducive to bulbil formation and underground

tuber proliferation. At the molecular level, this study found that Zhaotong City, Yunnan (C6), Guiyang City, Guizhou (C14), Mianyang City, Sichuan (C17), Chizhou City, Anhui (C7), and Yiwu City, Zhejiang (C13) all exhibited high genetic diversity, representing distribution areas of wild superior germplasm.

In addition to breeding superior varieties or cultivating new varieties of *P. ternata*, conservation measures should be implemented for its wild resources, with in-situ or ex-situ conservation applied to areas with high genetic diversity. Due to its wide distribution but small wild population size, ex-situ conservation should be the primary approach, such as establishing germplasm resource gardens (banks) for medicinal plant *P. ternata*, focusing on protecting populations with high genetic diversity. Populations possessing 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 also receive priority protection. Meanwhile, when developing ex-situ conservation strategies, individuals with special traits should be included in the conservation scope as much as possible.

References

AN Y, YANG D, LI X, et al., 2018. Study on the obstacle effect and physiological mechanism of *Pinellia ternate* continuous cropping[J]. J NW Agric, 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, FORSETER 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 Re, 27: 2449-2455.

CHANG Q, 2022. Genetic diversity analysis and excellent germplasm selection of *Pinellia ternate*[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.

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.

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 hypolytypes[J]. Nat Sci J Harbin Norm Univ, 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]. John Wiley & Sons Inc. 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.

HAFFER J, 1969. Speciation in amazonian forest birds[J]. Science (New York, N.Y.), 165(3889): 131-137.

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

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

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.

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, racL-a, trnL-trnF sequences[J/OL]. Mol Plant Breed: 1-14.

JIANG FJ, 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 YX, LIU XD, ZHU ZJ, 2004. Progress on the research of pharmacology of *Pinellia ternate*[J]. J Liaoning Univ Tradit Chin Med, 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.

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

LU XL, 2018. Phylogeography of *Camellia nitidissima*[D]. Guilin: Guangxi Normal University: 1-61.

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.

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 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.

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.

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.

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: China Academy of Forestry Sciences: 1-132.

TAJJMA 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 ternate* geimplasm[J]. Guizhou Agric Sci, 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): 4104-4115.

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.

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.

ZHANG JY, 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 region in China[J]. Chin J Chin Mat Med, 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 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 YF, 2022. Application of chloroplast genome in botany research[J]. Biol Teach, 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.

ZHAO YM, LI XL, 2016. An application of matK and rbcL in *Pinellia*[J]. J Shangluo Univ, 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.

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