AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-202003.00060

Postprint: Genetic Diversity and Population Historical Dynamics of *Eupatorium heterophyllum*

Authors: Pan Yuezhi, ZHAO Yujuan, Gong Xun

Date: 2020-03-24T00:00:00+00:00

Abstract

Eupatorium heterophyllum belongs to the genus Eupatorium in the family Asteraceae and is a species distributed at relatively high altitudes within this genus, occurring in the eastern Qinghai-Tibet Plateau and the Hengduan Mountains at elevations of approximately 1,700–3,000 m. This study employed two chloroplast DNA (cpDNA) fragments, ycf6-psbM and rpl32-trnL, as well as the nuclear DNA fragment ITS (nITS) as molecular markers to investigate the genetic diversity and distribution characteristics of Eupatorium heterophyllum, while also exploring its population historical dynamics. Combined analysis of chloroplast fragments revealed a haplotype diversity index (Hd) of 0.656 and nucleotide polymorphism ( ) of 0.001 61; whereas ITS showed a genotype polymorphism index (Hd) of 0.687 and nucleotide polymorphism of 0.002 35. Therefore, Eupatorium heterophyllum exhibits relatively low genetic diversity at the species level. Both cpDNA and nITS analyses demonstrated that the total genetic diversity at the population level of Eupatorium heterophyllum is greater than the average genetic diversity within populations, with genetic variation occurring primarily among populations, and significant genetic differentiation existing between populations (cpDNA: Gst = 0.679, Nst = 0.655, FST = 0.655; nITS: Gst = 0.543, Nst = 0.370, FST = 0.584). However, since the Nst value is smaller than the Gst value, the distribution of Eupatorium heterophyllum does not exhibit a distinct phylogeographic structure. Based on the geographic distribution of haplotypes and Network analysis, it is inferred that the southern Hengduan Mountains (southwestern Sichuan–northwestern Yunnan) and central Yunnan may have served as two refugia for Eupatorium heterophyllum during the Quaternary glacial period, with results from neutrality tests and mismatch analysis supporting that no population expansion occurred in this species after the glacial period.

ChinaRxiv

Full Text

Preamble

Study on Genetic Diversity and Population Demography of *Eupatorium heterophyllum* (Asteraceae)

PAN Yuezhi, ZHAO Yujuan, GONG Xun*

Yunnan Key Laboratory for Wild Plant Resources, Department of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

---

Abstract

Eupatorium heterophyllum (Asteraceae) is distributed in the eastern Qinghai-Tibet Plateau and Hengduan Mountain regions at relatively high elevations compared to other species within this genus. This study investigated the genetic diversity, distribution patterns, and population historical dynamics of *E. heterophyllum* using two chloroplast DNA (cpDNA) fragments (ycf6-psbM and rpl32-trnL) and one nuclear DNA fragment (ITS, nITS) as molecular markers. Combined cpDNA analysis revealed a haplotype diversity index (Hd) of 0.656 and nucleotide diversity ( ) of 0.00161 at the species level, while ITS showed a genotypic diversity index (Hd) of 0.687 and nucleotide diversity of 0.00235. These results indicate relatively low genetic diversity in *E. heterophyllum* at the species level. Both cpDNA and ITS analyses demonstrated that total genetic diversity was greater than the average within-population diversity, with genetic variation occurring primarily among populations. Significant genetic differentiation was detected among populations (cpDNA: Gst = 0.679, Nst = 0.655, FST = 0.655; nITS: Gst = 0.543, Nst = 0.370, FST = 0.584). However, the absence of a clear phylogeographic structure was indicated by Nst values being smaller than Gst values. Based on haplotype geographic distribution and network analysis, we propose that southern Hengduan Mountains (southwestern Sichuan-northwestern Yunnan) and central Yunnan may have served as two refugia for *E. heterophyllum* during the Quaternary glaciation. Neutral tests and mismatch analysis support that no post-glacial population expansion occurred in this species.

Keywords: *Eupatorium heterophyllum*, genetic diversity, population demography, Hengduan Mountains, refugium

---

Introduction

Eupatorium heterophyllum belongs to the genus *Eupatorium* (Asteraceae). This genus was once considered a large cosmopolitan genus with approximately 600 species distributed across the Americas and Eurasia, including the notorious invasive species previously known as *E. adenophora* (Lin et al., 1985; Chen et al., 2011). However, taxonomic revision redefined *Eupatorium* as a small Arctic-Tertiary genus confined to Eurasia with only about 45 species (King & Robinson, 1970). Consequently, taxa from Central and South America were excluded, such as the former *E. adenophora*, which was reassigned to the genus *Ageratina* as *Ageratina adenophora* (King & Robinson, 1970; Chen et al., 2011). The redefined *Eupatorium* represents a north-temperate group with 27 species in North America, 25 in East Asia, and one in Europe, exhibiting a typical East Asian-North American disjunct distribution pattern (Schilling et al., 1999; Ito et al., 2000). Approximately 14 species occur in mainland China and Taiwan, including six endemics (Chen et al., 2011). Phylogenetic and biogeographic analyses suggest a North American origin, with one lineage dispersing to Eurasia via the Bering land bridge during the Late Tertiary (6.18–11.6 Ma) and subsequently undergoing radiation in Asia (Schilling et al., 1999; Schmidt & Schilling, 2000). East Asian species show relatively minor morphological and karyotypic differences (Watanabe et al., 1990), suggesting potential natural hybridization (Schmidt & Schilling, 2000).

Among Chinese species, *E. heterophyllum* is uniquely distributed at higher elevations (1,700–3,000 m) in the eastern Qinghai-Tibet Plateau and Hengduan Mountains, inhabiting forest understories, forest margins, grasslands, and river valleys (Chen et al., 2011). Chemical composition varies among populations (Saito et al., 2014). The Hengduan Mountain region represents a floristic unit of the Pan-Arctic kingdom, characterized by a fundamentally temperate flora with strong north-temperate elements, many of which are Arctic-Tertiary taxa that migrated southward following dramatic Miocene cooling (Li & Li, 1993). The Qinling Mountains-Yellow River line along the Sichuan Basin likely served as the primary migration route for Arctic-Tertiary elements into the Himalaya-Hengduan region (Sun, 2002). During the Quaternary glaciation, this area remained free of unified ice sheets and was influenced by both East Asian and Indian monsoon climates, providing refugia for numerous plant lineages, particularly in lower-elevation zones (Qiu et al., 2011; Gengji et al., 2018). Postglacially, the Hengduan region became a crucial center of origin and radiation for modern temperate plants (Li & Li, 1993; Yu & Zhang, 2013), exhibiting exceptionally high species and genetic diversity (Yu et al., 2019).

As the only high-elevation species in *Eupatorium*, *E. heterophyllum* offers an opportunity to investigate its Quaternary population history and contemporary genetic diversity patterns. This study addresses these questions through sequencing of two cpDNA fragments (ycf6-psbM and rpl32-trnL) and the nuclear ITS region, employing population genetic and phylogeographic analytical approaches.

Materials and Methods

1.1 Plant Material

We collected leaf samples from 261 individuals across 27 populations in Gansu, Sichuan, Yunnan, and Guizhou provinces (Table 1 ; Figure 1 [Figure 1: see original paper], Figure 2 [Figure 2: see original paper]). Samples were silica-dried and transported to the laboratory for analysis.

1.2 DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted using the CTAB method (Doyle & Doyle, 1987). The nuclear ribosomal ITS region was amplified using primers ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) (White et al., 1990). Two cpDNA regions were amplified: ycf6-psbM using primers ycf6F (ATGGATATAGTAAGTCTYGCTTGGGC) and psbMR (ATGGAAGTAAATATTCTYGCATTTATTGCT) (Shaw et al., 2005), and rpl32-trnL using primers rpl32F (CAGTTCCAAAAAAACGTACTTC) and trnLR (CTGCTTCCTAAGAGCAGCGT) (Shaw et al., 2007).

PCR conditions for ITS consisted of initial denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, 53°C for 1 min, and 65°C for 1 min, with a final extension at 65°C for 7 min. The ycf6-psbM protocol was similar but with an annealing temperature of 52°C. For rpl32-trnL, amplification involved initial denaturation at 80°C for 2 min, followed by 33 cycles of 95°C for 1 min, 50°C for 1 min, and 65°C for 1.5 min, with final extension at 65°C for 5 min.

PCR products were purified using the W5211 purification kit (Sangon Biotech, Shanghai) and bidirectionally sequenced on an ABI3730 sequencer.

1.3 Data Analysis

Raw sequences were assembled using Seqman (DNAStar, Inc., Madison, USA) and aligned with Clustal X (Thompson et al., 1997). The two cpDNA fragments were concatenated in PAUP*4.0b10 (Swofford, 2002). DnaSP 5.10 (Librado & Rozas, 2009) was used to calculate haplotype number, variable sites, haplotype diversity (Hd), and nucleotide diversity ( ), and to perform neutrality tests (Tajima’ s D and Fu and Li’ s F)* to infer population size changes under expansion models. Geographic distributions of haplotypes were mapped using ArcGIS 10.2. Haplotype network relationships were constructed using Network 5.011 (http://www.fluxus-engineering.com).

Permut (Pons & Petit, 1996) was employed to calculate within-population genetic diversity (Hs), total genetic diversity (Ht), and inter-population differentiation coefficients (Gst and Nst). Analysis of molecular variance (AMOVA) was conducted using Arlequin 3.5 (Excoffier & Lischer, 2010) to assess genetic variation within and among populations. This software was also used to evaluate Fst for haplotype distributions and to perform Mantel tests (1,000 permutations) comparing geographic and genetic distance matrices. Additionally, mismatch distribution analysis was performed to compute the sum of squared deviations (SSD) and raggedness index (r) between observed and expected values under expansion models, along with their significance (P values).

Results

2.1 cpDNA Data Analysis

Successful sequencing of both ycf6-psbM and rpl32-trnL was obtained for 260 individuals. The concatenated alignment comprised 1,179 bp. DnaSP 5.10 identified 11 haplotypes. Population-level nucleotide diversity ( ) and haplotype diversity (Hd) values are presented in Table 1. At the species level, haplotype diversity (Hd) was 0.656 and nucleotide diversity ( ) was 0.00161.

Haplotype H1 occurred in 11 populations across northwestern Yunnan, southwestern Sichuan, Wenxian (Gansu), and Shibing (Guizhou), with minor presence in Kunming (population 24) and Dongchuan (population 23). Haplotype H4 was distributed in five central Yunnan populations. Haplotype H5 was exclusive to Kunming population 24, H9 to Yulong population 13 in Yunnan, and H10 to Dongchuan population 22 (Figure 1, Table 1). Network analysis positioned the most frequent haplotypes H1 and H4 centrally, with haplotypes H3, H10, H11, H8, H5, H6, and H5 at terminal positions (Figure 3 [Figure 3: see original paper]).

Population SSD and raggedness index values were non-significant positive values ($P > 0.05$). Neutrality tests yielded negative but non-significant Tajima' s D (-0.59) and Fu and Li' s F (-0.06) values ($P > 0.05$). The mismatch distribution curve was multimodal (Figure 5 [Figure 5: see original paper]), with observed values deviating from expectations under expansion models. These results collectively indicate no significant population expansion in *E. heterophyllum*.

Total genetic diversity (Ht) was 0.682, with average within-population diversity (Hs) of 0.219. Inter-population differentiation coefficients were Gst = 0.679 and Nst = 0.655 ($Nst < Gst$). AMOVA revealed that 65.55% of genetic variation occurred among populations, with only 34.45% within populations (Fst = 0.655). Genetic and geographic distances were positively correlated but marginally significant (r = 0.18, P = 0.045).

2.2 ITS Analysis

Successful ITS sequencing was obtained for 261 individuals, including 197 homozygotes and 64 heterozygotes. The aligned matrix length was 638 bp, which yielded 522 sequences after phasing with DnaSP 5.10. These sequences comprised 21 genotypes (H1-H21) (Figure 2, Table 1). Species-level genotype diversity (Hd) was 0.6869 with nucleotide diversity of 0.00235. Population-level diversity indices are shown in Table 1.

The most frequent genotypes were H1 and H3. Genotype H1 occurred in central Yunnan and in Mianning and Jiulong (Sichuan), with central Yunnan as its primary distribution. Genotype H3 ranged from Wenxian (Gansu) along the western edge of the Sichuan Basin to northwestern Yunnan and Shibing (Guizhou), being most abundant in northwestern Yunnan and southwestern Sichuan (Figure 2, Table 1). Genotype H2 occurred in populations 1, 12, 13, 14, 19, 24, and 27, showing a distribution pattern from northwestern Yunnan to central and north-central Yunnan and extending to Guizhou (population 27), excluding Wenxian (population 1). Genotypes H4 and H5 showed similar distribution patterns in Wenxian (population 1), northwestern Yunnan (population 14), and north-central Yunnan (population 19). Genotype H7 was restricted to three north-central Yunnan populations (20, 21, 22), while H11 occurred only in two north-central Yunnan populations (23, 25). Genotype H8 was found in southwestern Sichuan (population 16), northwestern Yunnan (populations 8, 13, 15), and Guizhou (population 27). Private genotypes included H6 (population 14), H10 (population 13), H12 and H13 (population 2), H15 (population 1), H16 and H17 (population 17), H18-H20 (population 18), and H21 (population 27). Thus, ITS genotypes exhibited two main distribution patterns: (A) a "Gansu-southwestern Sichuan-northwestern Yunnan-Guizhou" type represented by H3, and a "southwestern Sichuan-central Yunnan" type represented by H1; and (B) "Sichuan Basin margin" private genotypes such as H12, H13, and H15. High-frequency genotypes H1 and H3 occupied central positions in the network, which contained multiple reticulate loops indicative of nuclear gene recombination events (Figure 4 [Figure 4: see original paper]).

Population SSD and raggedness index values were non-significant positive values ($P > 0.01$). Tajima' s D was negative (-1.14) but non-significant ($P > 0.1$), while Fu and Li' s F was 0.64. The mismatch distribution curve was bimodal (Figure 5), with observed values deviating from expectations under expansion models. These findings suggest that populations have not experienced expansion but rather remain in dynamic equilibrium, consistent with cpDNA results.

Total genetic diversity (Ht) was 0.712, with average within-population diversity (Hs) of 0.325. Inter-population differentiation coefficients were $Gst = 0.543$ and $Nst = 0.370$ ($Nst < Gst$). AMOVA indicated that 58.44% of genetic variation occurred among populations and 41.56% within populations ($Fst = 0.584$). Mantel tests revealed a significant positive correlation between genetic and geographic distances ($r = 0.43$, $P = 0$).

Discussion

3.1 Genetic Diversity and Structure of *Eupatorium heterophyllum*

Our analysis of three cpDNA fragments and ITS from 261 individuals across 27 populations identified 11 cpDNA haplotypes and 21 ITS genotypes, showing relatively high haplotype diversity. However, nucleotide diversity at the species level was low (cpDNA: = 0.00161; ITS: = 0.00235). For comparison, *Tugarinovia mongolica* (Asteraceae), a monotypic genus from Inner Mongolia, exhibited much higher nucleotide diversity (= 0.0092) and haplotype diversity (Hd = 0.9086) based on two cpDNA fragments, with clear differentiation between varieties (Zhao et al., 2019). *Leucomeris decora*, a deciduous small tree in Asteraceae, showed = 0.00102 for combined cpDNA data (trnQ-rps16, rpl16, rpl32-trnL) and = 0.00237 for the nuclear GAPDH fragment (Zhao & Gong, 2012). *Ligularia hodgsonii*, a Sino-Japanese disjunct Asteraceae species distributed around the Sichuan Basin, had = 0.00299 and Hd = 0.847 based on three cpDNA fragments (Wang et al., 2013). Thus, *E. heterophyllum* exhibits relatively low nucleotide and haplotype diversity compared to these congeners, though ITS data revealed relatively high haplotype diversity in several Sichuan Basin margin populations (Table 1, Figure 2).

Both cpDNA (Ht = 0.764, Hs = 0.250) and ITS (Ht = 0.746, Hs = 0.382) data showed that total genetic diversity exceeded average within-population diversity. AMOVA confirmed higher inter-population than intra-population variation (cpDNA: Fst = 0.655; ITS: Fst = 0.584). Permut analysis revealed high differentiation coefficients (cpDNA: Gst = 0.679, Nst = 0.655; ITS: Gst = 0.543, Nst = 0.370). Petit et al. (2005) reported mean Gst values of 0.637 for maternally inherited markers and 0.184 for biparentally inherited markers across 124 and 77 angiosperm species, respectively. *Eupatorium heterophyllum* exceeds both values, indicating pronounced genetic differentiation among populations. However, Nst < Gst suggests no clear phylogeographic structure (Pons & Petit, 1996). Nevertheless, haplotype distribution patterns reveal two relatively distinct regions: a "Gansu-southwestern Sichuan-northwestern Yunnan-Guizhou" area and a "central Yunnan" area, each harboring private and dominant haplotypes. The haplotype network shows that haplotypes from these regions do not form separate clades, and low-frequency shared haplotypes occur between them. As a perennial herb with strong adaptability and effective long-distance seed dispersal, *E. heterophyllum* likely maintains substantial gene flow between these regions, shaping its current genetic diversity pattern.

3.2 Population Historical Dynamics of *Eupatorium heterophyllum*

A primary objective of phylogeography is to infer glacial refugia and post-glacial range expansions (Avise, 2000; Liu et al., 2012). The eastern Qinghai-Tibet Plateau and southwestern China harbor numerous ancient and newly originated species, many of which expanded their ranges post-glacially (Liu et al., 2012; Qiu et al., 2011), though some, like *Primula secundiflora*, did not (Wang et al., 2008). For *E. heterophyllum*, neutrality tests and mismatch analysis indicate no population expansion.

Glacial refugia typically retain high genetic and haplotype diversity, with haplotype distributions reflecting geographic patterns (Hewitt, 1996, 2000; Petit et al., 2003). Coalescent theory suggests that high-frequency, centrally positioned haplotypes in networks are ancestral, while private haplotypes represent recent derivatives (Emerson et al., 2001). Network analysis places cpDNA haplotypes H1 and H4 and ITS haplotypes H1 and H3 in central positions with broad geographic distributions, suggesting they are ancestral. CpDNA haplotype H1 and ITS haplotype H3 are widespread across the Gansu-southwestern Sichuan-northwestern Yunnan region and Guizhou, while cpDNA haplotype H4 and ITS haplotype H1 are distributed in central Yunnan. We therefore hypothesize that southern Hengduan Mountains (southwestern Sichuan-northwestern Yunnan) and central Yunnan served as two refugia for *E. heterophyllum* during Quaternary glaciations, particularly the Last Glacial Maximum.

References

Avise JC. 2000. *Phylogeography: The History and Formation of Species*. Cambridge, Massachusetts: Harvard University Press.

Chen Y, Takayuki K, Hind DJN. 2011. Tribe Eupatorieae. In: Wu ZY, Raven PH, Hong DY (eds). *Flora of China* (Volumes 20-21). Beijing: Science Press; St. Louis: Missouri Botanical Garden Press. p. 879-891.

Crandall KA, Templeton AR. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, 134(3): 959-969.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation method for small quantities of fresh tissues. *Phytochemical Bulletin*, 19: 11-15.

Emerson BC, Paradis E, Thebaud C. 2001. Revealing the demographic histories of species using DNA sequences. *Trends in Ecology & Evolution*, 16: 707-716.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10: 564-567.

Gengji Z, Li Y, Jia L, et al. 2018. Phylogeography of *Saxifraga tangutica* Engl. (Saxifragaceae). *Acta Botanica Boreali-Occidentalia Sinica*, 38(2): 370-380.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, 58(3): 247-276.

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

Ito M, Watanabe K, Kita K, et al. 2000. Phytogeography of *Eupatorium* (Eupatorieae, Asteraceae): Insights from sequence data of the nrDNA regions and cpDNA RFLP. *Journal of Plant Research*, 113: 79-89.

King RM, Robinson H. 1970. *Eupatorium*, a composite genus of Arcto-Tertiary distribution. *Taxon*, 19: 769-774.

Librado P, Rozas J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25: 1451-1452.

Li XW, Li J. 1993. A preliminary floristic study on the seed plants from the region of Hengduan Mountain. *Acta Botanica Yunnanica*, 15(3): 217-231.

Lin R, Chen YL, Shi T. 1985. Compositae (1). In: *Flora Reipublicae Popularis Sinicae*, Vol. 74. Beijing: Science Press. p. 54-69.

Liu JQ, Sun YS, Ge XJ, et al. 2012. Phylogeographic studies of plants in China: Advances in the past and directions in the future. *Journal of Systematics and Evolution*, 50(4): 267-275.

Petit RJ, Aguinagalde I, Beaulieu JL, et al. 2003. Glacial refugia: Hotspots but not melting pots of genetic diversity. *Science*, 300(5625): 1563-1565.

Petit RJ, Duminil J, Fineschi S, et al. 2005. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology*, 14(3): 689-701.

Pons O, Petit RJ. 1996. Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, 144(3): 1237-1245.

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

Saito Y, Mukai T, Iwamoto Y, et al. 2014. Germacranolides and their diversity of *Eupatorium heterophyllum* collected in P.R. China. *Chemical & Pharmaceutical Bulletin*, 62(11): 1092-1099.

Schilling EE, Panero JL, Cox PB. 1999. Chloroplast DNA restriction site data support a narrowed interpretation of *Eupatorium* (Asteraceae). *Plant Systematics and Evolution*, 219: 209-223.

Schmidt GJ, Schilling EE. 2000. Phylogeny and biogeography of *Eupatorium* (Asteraceae: Eupatorieae) based on nuclear ITS sequence data. *American Journal of Botany*, 87(5): 716-726.

Shaw J, Lickey EB, Beck J, et al. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast sequences for phylogenetic analysis. *American Journal of Botany*, 92(1): 142-166.

Shaw J, Lickey EB, Schilling EE, et al. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany*, 94(3): 275-288.

Sun H. 2002. Evolution of Arctic-Tertiary flora in Himalaya-Hengduan Mountain. *Acta Botanica Yunnanica*, 24(6): 671-688.

Swofford DL. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods*), Version 4.0b10. Sunderland, MA: Sinauer Associates.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, 132(2): 619-633.

Thompson JD, Gibson TJ, Plewinak F, et al. 1997. The Clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25: 4876-4882.

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

Wang JF, Gong X, Chiang YC, et al. 2013. Phylogenetic patterns and disjunct distribution in *Ligularia hodgsonii* Hook. (Asteraceae). *Journal of Biogeography*, 40: 1741-1754.

Watanabe K, Ito M, Yahara T, et al. 1990. Numerical analyses of karyotypic diversity in the genus *Eupatorium* (Compositae, Eupatorieae). *Plant Systematics and Evolution*, 170: 215-228.

White TJ, Bruns T, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (eds). *PCR Protocols: A Guide to Methods and Applications.* San Diego: Academic Press. p. 315-322.

Yu H, Favre A, Sui X, et al. 2019. Mapping the genetic patterns of plants in the region of the Qinghai-Tibet Plateau: Implications for conservation strategies. *Diversity and Distributions*, 25: 109-107.

Yu H, Zhang Y. 2013. Advances in phylogeography of alpine plants in the Tibetan Plateau and adjacent regions. *Acta Botanica Boreali-Occidentalia Sinica*, 33(6): 1268-1278.

Zhao Y, Gong X. 2012. Genetic structure of the endangered *Leucomeris decora* (Asteraceae) in China inferred from chloroplast and nuclear DNA markers. *Conservation Genetics*, 13: 271-281.

Zhao Y, Pan B, Zhang M. 2019. Phylogeography and conservation genetics of the endangered *Tugarinovia mongolica* (Asteraceae) from Inner Mongolia, Northwest China. *PLoS ONE*, 14(2): e0211696.

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

Source: ChinaXiv —Machine translation. Verify with original.