

Visualizing Patterns of Genetic Landscapes and Species Distribution of *Taxus wallichiana* (Taxaceae), Based on GIS and Ecological Niche Models

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Abstract: The Chinese yew (*Taxus wallichiana*), which is widely distributed in the Himalayas and in southern China, is now on the edge of extinction. In order to understand the evolutionary processes that control the current diversity within this species at the genetic and ecological levels, its genetic patterns and range dynamics must first be identified and mapped. This knowledge can then be applied in the development of an effective conservation strategy. Based on molecular data obtained from 48 populations of *T. wallichiana*, we used GIS-based interpolation approach for the explicit visualization of patterns of genetic divergence and diversity, and a number of potential evolutionary hotspots have been specifically identified within the genetic landscape maps. Within the maps of genetic divergence and diversity, five areas of high inter-population genetic divergence and six areas of high intra-population genetic diversity have been highlighted in a number of separate mountain regions, and these evolutionary hotspots should have the priority to be protected. Furthermore, four geographical barriers have been identified: the eastern Himalayas, the Yunnan Plateau, the Hengduan Mountains and the Taiwan Strait. According to ecological niche modeling (ENM), the populations of *T. wallichiana* within the Sino-Himalayan Forest floristic subkingdom experienced westward expansion from the periods of Last Inter-glacial to Last Glacial Maximum (LGM). Following the LGM, the distribution range overall became reduced and fragmented. These findings challenge the classic mode of contraction-expansion in response to the last glaciation. In conclusion, our findings suggest that the changes in geographical landscapes and climate that occurred during the Quaternary resulted in current genetic landscape patterns.

Key words: genetic landscape; phylogeography; GIS; Ecological Niche Models (ENMs); Himalayas

1 Introduction

It is well known that global climate fluctuations, especially the climatic oscillations of the Quaternary period, resulted in repeated glacial-postglacial cycles that dramatically shaped the contemporary distributions and genetic structures of many temperate plant species (Hewitt 2000, 2004). The cyclical expansion-contraction reflection of plant populations in response to repeated drastic climatic changes, and the subsequent genetic drift and founder effect, generated genetic variation at intra-specific level (e.g. Zhang *et al.* 2005; Yuan *et al.* 2008; Cun and Wang 2010). Meanwhile, existing glacial refugia, together with increasingly proved cryptic

(or micro-) refugia during the Quaternary were becoming the habitats where plant species retreated, persisted, and that harbored ancient haplotypes and high genetic diversity (Hewitt 2000). Besides being affected by the Quaternary climatic oscillations, the genetic structures of plants were also affected by geographical landscapes (Schönswetter *et al.* 2005; Zhang *et al.* 2011b). Landscape features were likely to promote range fragmentation, vicariance, and population isolation, which caused genetic divergence among populations (Bittkau and Comes 2008; Gao *et al.* 2012). By tracing the genealogical distribution of genetic variation, phylogeographic researches can reveal inter-population genetic divergence and intra-population genetic

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diversity, and may be able to infer the location of refugia (Avice 2000; Hickerson *et al.* 2010). Furthermore, the merging of genetic and geospatial data between the fields of phylogeography and landscape genetics, using mainly GIS-based methods, is more convincing than individual phylogeography or landscape genetics approaches in bringing explicit visualization, and in understanding the evolutionary processes that were affected by landscape features and past climate changes. Therefore, the integration of GIS-based methods into phylogeography can link macro-landscape features with micro-genetic process to reveal evolutionary history of species (Manel *et al.* 2003; Kidd and Ritchie 2006; Carstens and Richards 2007; Manel and Segelbacher 2009). In recent years, several powerful tools developed using GIS-based methods or data have played an important role in many fields of biogeography, notably the Dispersal Networks tool that can visualize dispersal corridors and identify biogeographic barriers (Chan *et al.* 2011), and ecological niche models (ENMs) that can use GIS-based environmental variables in the modeling of species distribution (e.g. Richards *et al.* 2007; Kozak *et al.* 2008; Zhang *et al.* 2011a; Schorr *et al.* 2012).

Although traditional phylogeographic analysis is unable to reveal explicit genetic patterns at the spatial level, nevertheless several spatial analytical approaches have been applied in visualizing intra-specific variation (Thomassen *et al.* 2010). Thus, Vandergast *et al.* (2011) have developed a GIS-based approach that can map patterns of genetic divergence and diversity, and have applied this approach across multiple species in southern California and Deserts of Mojave and Sonoran, in order to identify some evolutionary hotspot areas for conservation (Vandergast *et al.* 2008; Wood *et al.* 2012). Therefore, considering visualizing patterns of genetic divergence and diversity of species, the GIS-based method can be useful and effective in revealing important information relating to evolution and conservation, especially in regard to the endangered species.

Taxus wallichiana is the most widespread *Taxus* species in the Himalayas and in southern China, and is usually distributed in small discrete populations. It is an economically important tree with valuable cancer-inhibitory properties. In recent years, owing to overexploitation and human disturbance, this well-known indigenous species have diminished sharply and become fragmented, and it is now seriously threatened with extinction in China. For this reason, it was listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; available from <http://www.cites.org/eng/app/appendices.php>) in 1995 and within the Grade I category of National Protected Plant in China (available from <http://jky.qz.edu.cn/zhsj/gjzdbhz.htm>). Therefore, the protection of this threatened species is extremely urgent.

According to Chinese phytogeography, most regions of China are located within the East Asiatic Floristic Kingdom, which can be further divided into three subkingdoms: (i)

the Tibetan Plateau (TP) floristic subkingdom; (ii) the Sino-Himalayan Forest floristic subkingdom; and (iii) the Sino-Japanese Forest floristic subkingdom (Wu and Wu 1998). The latter two subkingdoms, which are the origin of a high level of biodiversity and endemism amongst temperate plant species, served as the most important ancient relict areas for temperate biota throughout the Quaternary (Wu and Wu 1998). An increasing amount of evidences from palaeo-data and phylogeographic studies have proved that both Quaternary environmental changes and geological factors exerted a major influence on the distribution and evolution of plants within the two subkingdoms (e.g. Lehmkuhl and Haselein 2000; Yu *et al.* 2000; Gao *et al.* 2007; Opgenoorth *et al.* 2010). In particular, in the TP and adjacent areas, uplift of the TP and Quaternary glaciations resulted in a rapid population differentiation and allopatric speciation of alpine plants (e.g. Li *et al.* 2012a; Gao *et al.* 2012). Likewise, as a result of fluctuations in the sea level, population fragmentation and admixture of temperate biota appeared in eastern China and on the island of Taiwan (e.g. Huang *et al.* 2001; Qiu *et al.* 2009). The Chinese yew is one of the few species that is present within all three floristic subkingdoms. In this work, we have selected it as a model organism to reveal how geographical landscapes and climatic changes during the Quaternary affected its evolutionary processes through visualizing patterns of genetic landscapes and species distribution.

2 Data and methods

2.1 Data source

Based on previous sampling information (Gao *et al.* 2007), 48 populations (a total of 1230 individuals) were chosen for this study, with each population comprising no fewer than 5 individuals. These populations were divided into three groups: the Sino-Himalayan Forest (SH) floristic subkingdom group, the Sino-Japanese Forest (SJ) floristic subkingdom group, and the Malesian (ML) floristic subkingdom group (Fig. 1; Table 1). Molecular data consisting of 19 cpDNA haplotypes sequences based on the *trnL-F* intron-spacer region were gathered from GenBank databases (accession numbers: EU052213-EU052231).

2.2 Data analysis

2.2.1 Genetic diversity and divergence analysis

Genetic diversity indices, including haplotype diversity (H_d) and nucleotide diversity (average sequence divergence among individuals, π_i) of 48 populations, and the net number of sequence differences among populations (D_A) (Nei and Li 1979) as the index of genetic divergence, were all calculated using the ARLEQUIN v3.5 package (Excoffier *et al.* 2005) under the Tamura and Nei model of nucleotide evolution (Tamura and Nei 1993). We also estimated the levels of gene flow amongst the three groups SH, SJ and ML using the ARLEQUIN v3.5 package.

Table 1 Geographic origins, sample sizes (N), haplotype diversity (H_d), and average sequence divergence among individuals (π_i) of 48 populations of *T. wallichiana*.

| Population code | Longitude (E) | Latitude (N) | Floristic subkingdom | N | H_d | π_i |
|-----------------|---------------|--------------|----------------------|----|--------|---------|
| GS | 98.38333333 | 27.70000000 | SH | 31 | 0.4538 | 0.3622 |
| DL | 100.05000000 | 25.71666667 | SH | 30 | 0.5356 | 0.1864 |
| JD | 100.73333333 | 24.36666667 | SH | 24 | 0.6920 | 0.1596 |
| YD | 99.50000000 | 24.25000000 | SH | 15 | 0.5619 | 0.0000 |
| QJ | 103.25000000 | 27.21666667 | SH | 31 | 0.0645 | 0.4659 |
| LJ | 100.15000000 | 27.01666667 | SH | 30 | 0.0000 | 0.0000 |
| LD | 99.53333333 | 27.15000000 | SH | 30 | 0.0000 | 0.0000 |
| BM | 99.18333333 | 28.26666667 | SH | 30 | 0.0000 | 0.0000 |
| DQ | 98.80000000 | 28.20000000 | SH | 30 | 0.0000 | 0.0000 |
| LG | 100.80000000 | 27.61666667 | SH | 30 | 0.0000 | 0.0000 |
| KP | 99.00000000 | 27.63333333 | SH | 30 | 0.4966 | 1.4939 |
| YY | 101.20000000 | 28.08333333 | SH | 10 | 0.0000 | 0.0000 |
| EM | 103.51666667 | 29.53333333 | SH | 30 | 0.2874 | 2.3157 |
| PZ | 103.56666667 | 30.85000000 | SJ | 19 | 0.4094 | 0.4097 |
| SP | 104.21666667 | 32.63333333 | SJ | 31 | 0.0000 | 0.0000 |
| LS | 108.21666667 | 26.23333333 | SJ | 31 | 0.5032 | 0.5037 |
| FRB | 108.68333333 | 28.00000000 | SJ | 30 | 0.3310 | 0.3313 |
| FJS | 108.78333333 | 27.75000000 | SJ | 30 | 0.3011 | 0.7051 |
| SZ | 109.93333333 | 29.73333333 | SJ | 5 | 0.4000 | 1.2034 |
| LCH | 109.08333333 | 30.01666667 | SJ | 30 | 0.5172 | 0.5177 |
| ES | 109.51666667 | 29.95000000 | SJ | 15 | 0.3429 | 0.3432 |
| BK | 111.45000000 | 31.66666667 | SJ | 29 | 0.0690 | 0.2075 |
| SN | 110.35000000 | 31.45000000 | SJ | 10 | 0.0000 | 0.0000 |
| HX | 108.80000000 | 32.13333333 | SJ | 22 | 0.0000 | 0.0000 |
| CG | 107.26666667 | 32.80000000 | SJ | 31 | 0.0000 | 0.0000 |
| LB | 106.83333333 | 33.68333333 | SJ | 30 | 0.0000 | 0.0000 |
| TB | 107.08333333 | 33.85000000 | SJ | 33 | 0.0000 | 0.0000 |
| HL | 111.23333333 | 33.75000000 | SJ | 30 | 0.0000 | 0.0000 |
| SL | 113.31666667 | 35.75000000 | SJ | 30 | 0.0000 | 0.0000 |
| WT | 110.00000000 | 25.55000000 | SJ | 25 | 0.4200 | 0.4204 |
| GY | 110.95000000 | 25.41666667 | SJ | 31 | 0.2323 | 0.2325 |
| GJ | 110.20000000 | 24.23333333 | SJ | 30 | 0.3310 | 0.3313 |
| LZ | 112.38333333 | 25.13333333 | SJ | 32 | 0.5141 | 0.5146 |
| JX | 114.63333333 | 24.83333333 | SJ | 31 | 0.3613 | 0.3616 |
| FQ | 119.13333333 | 25.76666667 | SJ | 30 | 0.1862 | 0.1864 |
| WP | 116.16666667 | 25.18333333 | SJ | 30 | 0.3701 | 0.3705 |
| YH | 116.16666667 | 27.25000000 | SJ | 22 | 0.3680 | 0.3683 |
| FY | 117.51666667 | 29.53333333 | SJ | 29 | 0.4877 | 0.4881 |
| JG | 114.16666667 | 26.56666667 | SJ | 21 | 0.3238 | 0.3241 |
| JY | 117.65000000 | 27.41666667 | SJ | 30 | 0.4966 | 0.4970 |
| HSH | 118.16666667 | 30.11666667 | SJ | 30 | 0.5793 | 0.9950 |
| LA | 119.50000000 | 30.31666667 | SJ | 20 | 0.5316 | 0.4794 |
| TSH | 119.68333333 | 27.51666667 | SJ | 30 | 0.3310 | 0.3313 |
| TW | 121.20000000 | 24.80000000 | ML | 17 | 0.5294 | 1.0612 |
| MLP | 104.13333333 | 23.31666667 | ML | 20 | 0.1895 | 0.1897 |
| SC | 104.88333333 | 23.36666667 | ML | 24 | 0.3913 | 0.0000 |
| NV1 | 104.65000000 | 20.81666667 | ML | 14 | 0.0000 | 0.0000 |
| NV2 | 105.06666667 | 23.10000000 | ML | 7 | 0.0000 | 0.0000 |

Note: SH, Sino-Himalayan Forest; SJ, Sino-Japanese Forest; ML, Malesian.

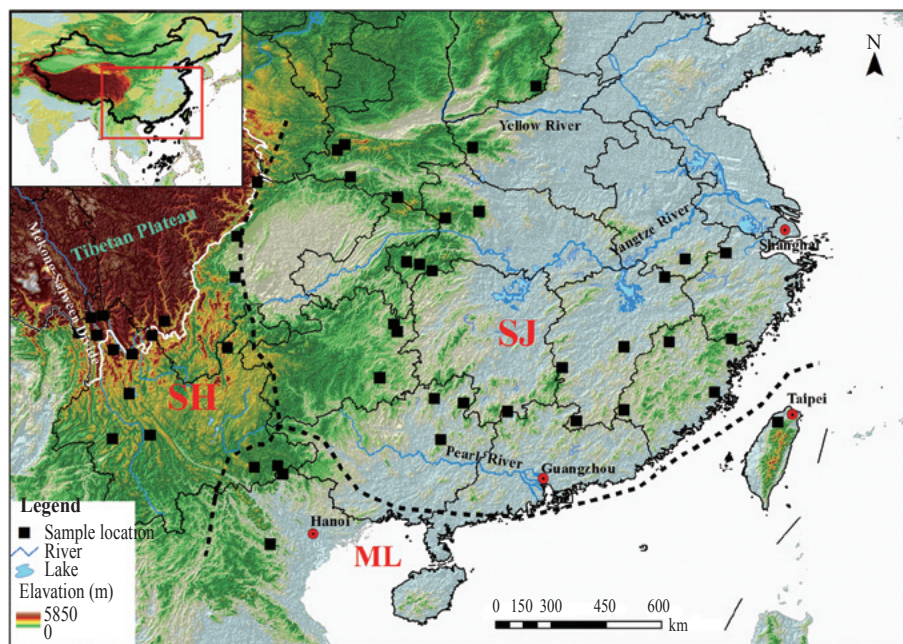


Fig. 1 Sample sites for *T. wallichiana*. The dotted lines indicate the approximate phytogeographical boundary of the East Asiatic Floristic Kingdom. SH, Sino-Himalayan Forest floristic subkingdom; SJ, Sino-Japanese Forest floristic subkingdom; ML, Malesian floristic subkingdom.

2.2.2 Spatial identification of geographical barriers

To examine the geographical barriers associated with genetic discontinuities among populations based on a network connected by Delaunay triangulation, BARRIER v2.2 software (Manni *et al.* 2004) was implemented using the Monmonier's maximum difference algorithm. Based on population locations and genetic divergence values (D_A), several higher genetic discontinuities were obtained, and the genetic discontinuities were then adjusted spatially in ArcGIS v9.3 (Environmental Systems Research Institute, Inc., Redlands, CA, USA).

2.2.3 Mapping genetic divergence and diversity patterns

We mapped patterns of genetic divergence and genetic diversity using the Genetic Landscape GIS Toolbox (Vandergast *et al.* 2011) in ArcGIS v9.3 (ESRI). Prior to this step, online IBDWS software (isolation by distance (IBD) web service at <http://ibdws.sdsu.edu/~ibdws/> (Jensen *et al.* 2005) was used to assess the significance of the relationship between genetic distances and geographic distances with a Mantel test and reduced major axis (RMA) regression analysis under 1000 random permutations (IBD analysis). The input data containing the pairwise divergence D_A matrix was obtained using the ARLEQUIN v3.5 package (Excoffier *et al.* 2005) and the geographical distance matrix was calculated using the Hawth's Tools extension in ArcGIS v9.3. Where IBD was significant, genetic divergence values (D_A) were mapped at the mid-points between populations and then a continuous surface (genetic divergence landscape, 1 km² grid cell size) were created using an inverse distance weighted interpolation algorithm. For the genetic diversity landscape, genetic diversity values (H_d and

π_i) were mapped at the population locations and interpolated in the same manner as the divergence layer (Vandergast *et al.* 2011).

2.2.4 Ecological niche modelling (ENM)

ENM was carried out to determine possible present and past species distributions of *T. wallichiana* using climate data and occurrence data. Climate data for three periods based on 19 bioclimatic variables (Table 2) were obtained from the WorldClim database (Hijmans *et al.* 2005), three periods were: the Last Interglacial (LIG; ~120–140 ka), the Last Glacial Maximum (LGM; ~21 ka), based on the Community Climate System Model (CCSM), and current conditions (~1950–2000). Species occurrence data were collected from the Chinese Virtual Herbarium (CVH; www.cvh.org.cn), Global Biodiversity Information Facility (GBIF; <http://data.gbif.org>) and field surveys. The present and past distributions of *T. wallichiana* were estimated using the maximum entropy algorithm in MAXENT v3.3 (Phillips *et al.* 2006), with the settings for convergence threshold (10^{-5}), number of iterations (500) and occurrence localities divided into testing data sets and training data sets (20% and 80%, respectively).

3 Results

3.1 Patterns of genetic divergence in *T. wallichiana*

An IBD analysis shows that there is a significant positive correlation between genetic distance (as measured by D_A) and geographic distance (km) according to a Mantel test ($r = 0.269$, $p < 0.001$). Additionally, RMA regression analysis suggests that 7.24% of the cpDNA variation amongst populations is explained by geographic distance ($y = -1.517 + 4.34 \times 10^{-3}x$, $R^2 = 0.0724$, $p = 0.107$) (Fig. 2a).

Table 2 Codes used for 19 bioclimatic variables.

| Code | Description | Code | Description |
|-------|--|-------|--|
| Bio1 | Mean annual temperature | Bio11 | Mean temperature of coldest quarter |
| Bio2 | Mean diurnal range (Mean of monthly [max temp–min temp]) | Bio12 | Annual precipitation |
| Bio3 | Isothermality (Bio2/Bio7) (*100) | Bio13 | Precipitation of wettest month |
| Bio4 | Temperature seasonality (standard deviation *100) | Bio14 | Precipitation of driest month |
| Bio5 | Max temperature of warmest month | Bio15 | Precipitation seasonality (Coefficient of Variation) |
| Bio6 | Min temperature of coldest month | Bio16 | Precipitation of wettest quarter |
| Bio7 | Temperature annual range (Bio5–Bio6) | Bio17 | Precipitation of driest quarter |
| Bio8 | Mean temperature of wettest quarter | Bio18 | Precipitation of warmest quarter |
| Bio9 | Mean temperature of driest quarter | Bio19 | Precipitation of coldest quarter |
| Bio10 | Mean temperature of warmest quarter | | |

In the divergence landscape map for *T. wallichiana*, areas of noticeable genetic divergence locate at the approximate boundary between pairs of floristic subkingdoms (Fig. 2c), and the estimates of gene flow occurring amongst the three floristic subkingdoms are low (Table 3). In addition, areas of remarkable genetic divergence also exist within the SH floristic subkingdom, where three hotspots of extremely

high genetic divergence (the eastern Himalayas, the Yunnan Plateau, and the Hengduan Mountains) are identified (Fig. 2c), and these three areas are identified as possible geographic barriers (lines *a*, *b* and *c*) by Monmonier's maximum difference algorithm (Fig. 2b). The other two hotspots of high genetic divergence are found in the mountain regions of southern China (Fig. 2c).

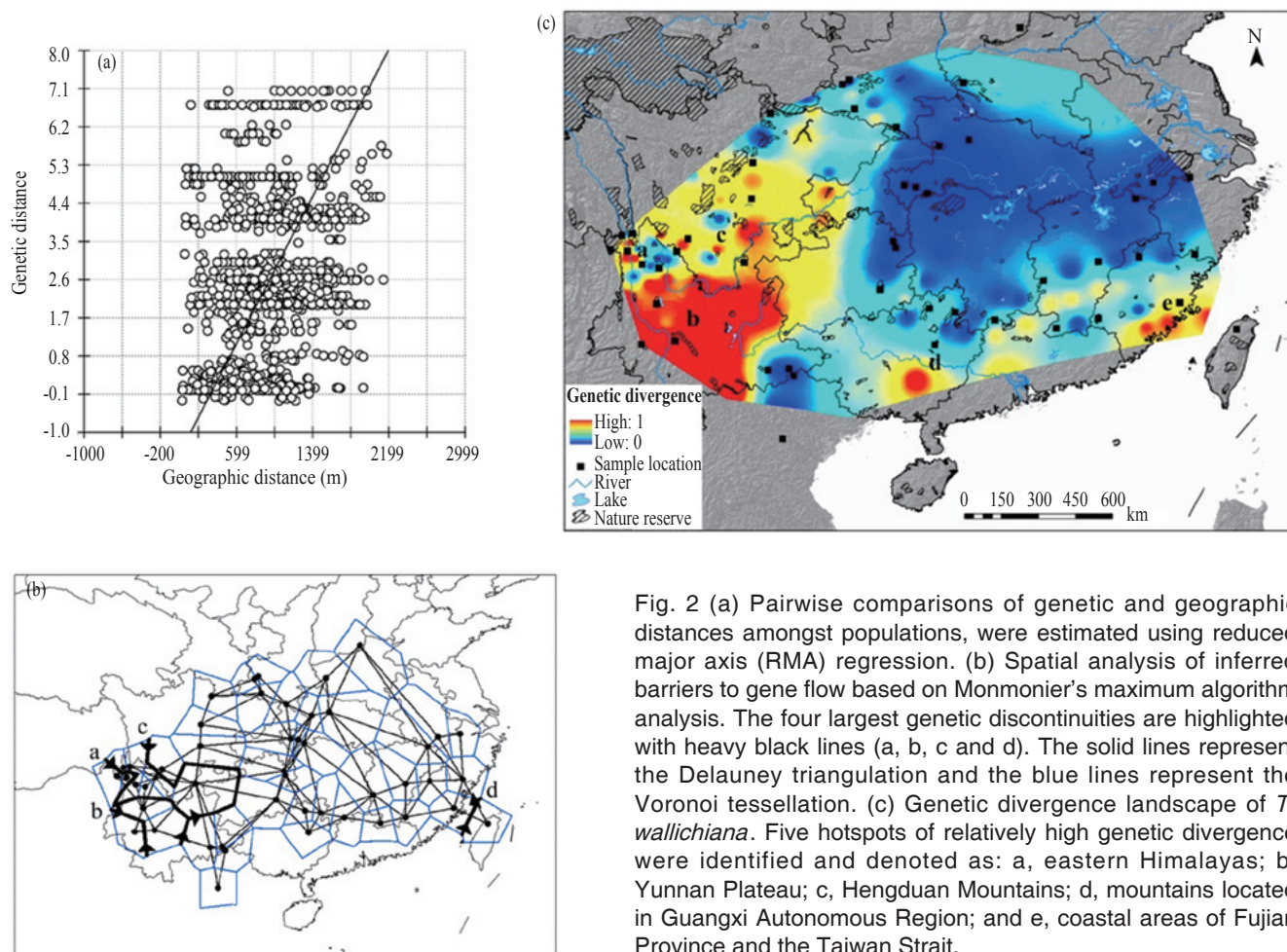


Fig. 2 (a) Pairwise comparisons of genetic and geographic distances amongst populations, were estimated using reduced major axis (RMA) regression. (b) Spatial analysis of inferred barriers to gene flow based on Monmonier's maximum algorithm analysis. The four largest genetic discontinuities are highlighted with heavy black lines (*a*, *b*, *c* and *d*). The solid lines represent the Delauney triangulation and the blue lines represent the Voronoi tessellation. (c) Genetic divergence landscape of *T. wallichiana*. Five hotspots of relatively high genetic divergence were identified and denoted as: *a*, eastern Himalayas; *b*, Yunnan Plateau; *c*, Hengduan Mountains; *d*, mountains located in Guangxi Autonomous Region; and *e*, coastal areas of Fujian Province and the Taiwan Strait.

3.2 Patterns of genetic diversity in *T. wallichiana*

The haplotype diversity (H_d) and nucleotide diversity (π_i) of 48 populations in *T. wallichiana* range from 0.00 to 0.69 and 0.00 to 2.32, respectively (Table 1). Based on the interpolation of two genetic diversity indices using ArcGIS v9.3, we obtained genetic diversity maps and have identified several areas of high haplotype diversity (H_d), including the eastern Himalayas, Yunnan Plateau and Mt. Huangshan, together with areas of high nucleotide diversity (π_i), such as the eastern Himalayas, the Hengduan Mountains, the Wuling Mountains, the Mt. Huangshan, and the island of Taiwan. In addition, we observe that low area of genetic diversity is located in the northern region of SJ floristic subkingdom (Fig. 3).

3.3 Ecological niche modeling (ENM)

We used the Maxent algorithm to simulate the potential distribution of *T. wallichiana* during the LIG, LGM and present-day periods (Fig. 4). The large area under the receiver operating characteristic (ROC) curve indicates a fine prediction (AUC>0.96). The species distributions for the three periods suggest that there have been little change in the predicted distributions, but we still observe that the populations of *T. wallichiana* underwent a westward expansion during the last glaciation. After the LGM, the range of *T. wallichiana* became reduced and sharply fragmented, appearing the pattern of discontinuous distribution of small populations that is seen during the current period.

Table 3 Estimates of gene flow amongst the three floristic subkingdoms.

| | SH | SJ | ML |
|----|---------|---------|----|
| SH | 0 | | |
| SJ | 0.38128 | 0 | |
| ML | 0.65477 | 0.39471 | 0 |

4 Discussion

4.1 Genetic divergence and diversity patterns

In the study reported here, we have obtained genetic divergence and diversity patterns for *T. wallichiana* using the powerful GIS-based approaches. In the genetic divergence map, the three floristic subkingdoms are individually identified (Fig. 2c), and most of the divergence hotspots are mainly concentrated within the SH floristic subkingdom, including the eastern Himalayas, the Yunnan Plateau, the Hengduan Mountains, which all possess the typical characteristics of high-altitude mountains and deep valleys. The three largest genetic discontinuities (equivalent to geographical barriers) also overlap the three floristic subkingdoms (Fig. 2b), and barrier formed by a and b belongs to a well-known geographic barrier, the “Mekong-Salween Divide”, which can effectively prevent an east-west mediated gene flow and thereby cause separate lineage divergence (e.g. Li *et al.* 2011). Within the SH floristic subkingdom, owing to the complexity of topology, landscape features have played an important role in the composition of the genetic structures of species, and in consequence, they have led to a high level of genetic divergence and diversity patterns for *T. wallichiana*. The

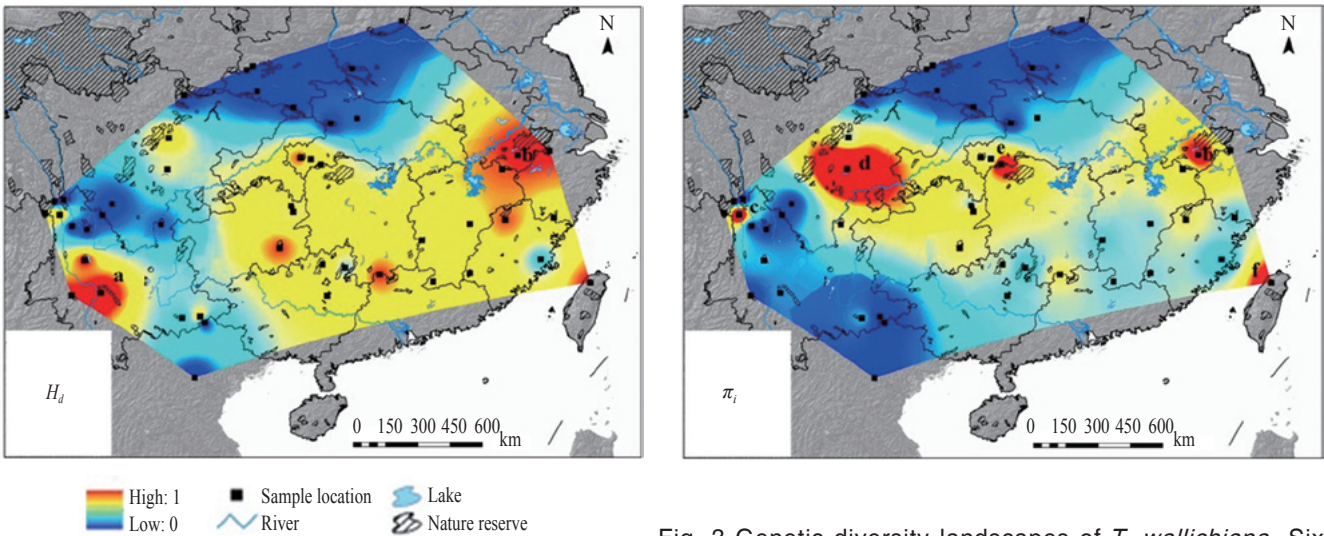


Fig. 3 Genetic diversity landscapes of *T. wallichiana*. Six areas of relatively high genetic diversity were identified and denoted as: a, Yunnan Plateau; b, Mt. Huangshan; c, eastern Himalayas; d, Hengduan Mountains; e, Wuling Mountains; and f, island of Taiwan.

similarity of genetic patterns has been found in a number of phylogeographic studies of alpine plants distributed within the SH floristic subkingdom (e.g. Yuan *et al.* 2008; Chen *et al.* 2008; Wang *et al.* 2008; Cun and Wang 2010). Aside from this subkingdom, sporadic areas of high genetic divergence and diversity are revealed in a number of mountains within both the SJ floristic subkingdom and ML floristic subkingdom (Fig. 2c; Fig. 3). The island of Taiwan, which belongs to ML floristic subkingdom, is usually regarded as a potential refugia for several gymnosperms, such as *Cunninghamia konishii*, *Taiwannia cryptomerioides*, which reached the island through land bridges from the mainland during the late Tertiary and Quaternary periods (Hwang *et al.* 2003; Chou *et al.* 2011). *T. wallichiana* was also estimated to arrive in Taiwan from the eastern part of SJ floristic subkingdom in the same period. Thereafter, it became isolated by the Taiwan Strait and then differentiated gradually as a result of glacial-interglacial cycles (Gao *et al.* 2007). Compared to the areas of high genetic diversity, the northern part of SJ floristic subkingdom shows uniformly low genetic diversity (Fig. 3). It is supposed that populations colonized of northern areas in the postglacial (or interglacial) periods with genetic diversity being reduced due to genetic drift and founder effects. Evidences for such northward colonization are supported by a few of temperate deciduous forest species (e.g. *Ginkgo biloba*, *Pteroceltis tatarinowii*) (Gong *et al.* 2008; Li *et al.* 2012b).

4.2 The mode of response to the last glaciation

It is of course well known that the European and North American continents were covered by a continuous ice sheet during the LGM, the classical glacial “*tabula rasa*” scenario for plants was well applied into the both continents in describing the response to the last glaciation (e.g. Taberlet *et al.* 1998; Soltis *et al.* 2006), which had a great impact on the distributions and genetic structures of many temperate plant species in these regions. Although no great ice sheet appeared in the TP during the Quaternary, cycles of glaciation still affected the species distributions of

alpine plants, especially in the case of the largest glaciation (~800–600 ka BP), which exerted the greatest influence (Cun and Wang 2010; Gao *et al.* 2012). With regard to the responses of alpine plants to the last glaciation, regional distinctions can be recognized in different parts of TP. In the northeastern part of TP, the dominant coniferous trees, *Juniperus przewalskii* and *Picea crassifolia*, are representative of a contraction mode that occurred in response to the last glaciation (Zhang *et al.* 2005; Meng *et al.* 2007), whereas, completely contrary to the plants mentioned above, the Chinese yew had the reflection of populations’ expansion in the southern edge of TP during the last glaciation (Fig. 4). This distinctive expansion mode was also supported by another coniferous species, *Picea likiangensis* within the Hengduan Mountains (Li *et al.* 2013). During the last glaciation, when the climate turned wetter and colder, and in consequence *T. wallichiana* was well adapted to the prevailing conditions, it could have migrated downwards and westwards and thereby expanded into larger areas of suitable habitats. Following the LGM, however, the distribution ranges for Chinese yew contracted into small populations (Fig. 4), and there is no doubt that climatic warming, and more recently, human overexploitation are the main reasons accounting for its current distribution.

4.3 Implications of potential evolutionary hotspots for conservation

In view of the overexploitation of *T. wallichiana* in recent years, it is urgent to the investigation of the level of genetic diversity for this endangered species and the development of proper conservation strategies are now urgent priorities, and genetic diversity is a significant factor in overall biodiversity protection (Noss 1990). Based on patterns of genetic diversity, six areas of high intra-population genetic diversity have been highlighted (Fig. 3), and most of them are focused on the SH floristic subkingdom, which is generally regarded as a major refugia of Quaternary glaciation, and which has facilitated the survival of biota

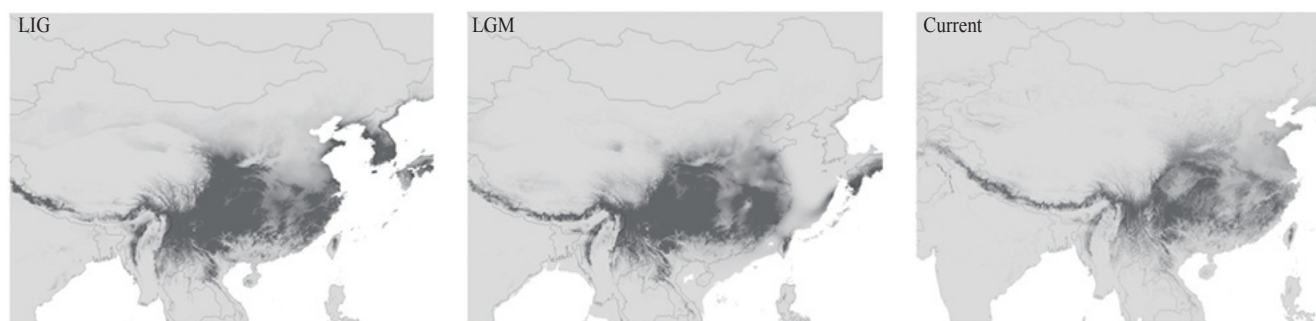


Fig. 4 The potential distribution of *T. wallichiana* during three historical periods, as simulated by the Maxent algorithm. The areas of greatest suitability are represented by dark shading. LIG, Last Interglacial; LGM, Last Glacial Maximum.

and has harbored high levels of genetic diversity (Qiu *et al.* 2011; Keppel *et al.* 2011). An optimal conservation planning must therefore include the prior establishment of nature reserves which contain hotspots of genetic diversity and the introduction and enforcement of strict laws to prevent wild resources from being exploited (*in situ* conservation). Currently, the fact that several nature reserves are quite small and fragmented (Fig. 3), together with the presence of a number of hydropower stations that are built over the major rivers, and which could possibly change the local ecological environment and affect the Chinese yew distributions, should be aroused enough attention. On the other hand, although estimates of the average gene diversity within the population (H_s) and of the total gene diversity amongst all populations (H_T) of *T. wallichiana* have been found to be relatively high (Gao *et al.* 2007), there were nevertheless low areas of genetic diversity associated with the populations of northern China and Vietnam. In these low areas of genetic diversity, the existing populations must be strictly preserved against any kind of exploitation, and the transplanting of individuals from genetically more diverse populations might be undertaken in order to broaden the genetic diversity. In addition, *ex situ* conservation initiatives, including the development of tissue culture technique, the establishment of seed bank from all the populations and the setting up of plantation bases, are also feasible conservation strategies. In any event, the *in situ* and *ex situ* conservation that are adopted need to strike the right balance between the conservation of genetic diversity required for the sustainable evolution of *T. wallichiana* in its natural populations and the proper exploitation and utilization of this species as a medical resource for human benefit.

5 Conclusion

Many phylogeographic studies of alpine plants have proved that geographical landscapes resulting from the TP uplift and the subsequent climatic changes in the Quaternary are the main factors accounting for high levels of genetic divergence and diversity patterns (Qiu *et al.* 2011; Yu and Zhang 2013). In this study, the analysis of patterns of genetic landscape and species distribution for *T. wallichiana* also support this conclusion. Within the SH subkingdom, *T. wallichiana* expanded its range during the LGM, contrary to the classic mode of population contraction-expansion in response to the last glaciation. Nevertheless, additional evidences are required for cold-adapted species in order to agree with the response mode in this region. Looking to the future, faced with climate warming and overexploitation of *T. wallichiana*, both *in situ* and *ex situ* conservation strategies must now be considered. Finally, by incorporating GIS-based methods for spatial analysis into phylogeographic studies, not only have we converted genetic data into a spatial display, but we have also been able to identify potential evolutionary hotspots, possible

secondary suture zones of hybridization and dispersal routes. The visualization of patterns of genetic divergence and diversity will therefore be a promising additional application for future phylogeographic studies.

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基于GIS和生态位模型的西藏红豆杉遗传景观和物种分布格局

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摘 要: 广泛分布于喜马拉雅山脉和我国华南地区的西藏红豆杉面临灭绝, 揭示空间遗传结构和物种分布变化对于理解西藏红豆杉的进化过程及其物种保护尤为重要。基于西藏红豆杉48个种群的分子变异数据, 利用反距离权重空间差值方法得到遗传多样性和遗传分化分布图, 识别出6个遗传多样性和5个遗传分化高值区域, 这些重点区域主要位于我国南方的几个山区, 这些区域在未来应给予重点保护; 基于Monmonier算法识别出4条地理隔离: 东喜马拉雅山脉、横断山脉、云南高原和台湾海峡; 利用生态位模型模拟三个历史时期的物种分布格局, 从末次间冰期至末次冰盛期, 物种经历一次向高原西部的面积扩张, 这与典型的冰期物种退缩模式不同; 末次冰盛期之后, 物种面积逐渐缩小, 呈现破碎化分布。因此, 地理景观特征和第四纪气候波动对西藏红豆杉遗传结构和分布格局影响很大。

关键词: 遗传景观; 谱系地理学; 地理信息系统; 生态位模型; 喜马拉雅山脉