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Phylogeography of *Cephalotaxus oliveri* (Cephalotaxaceae) in relation to habitat heterogeneity, physical barriers and the uplift of the Yungui Plateau

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ABSTRACT

Habitat heterogeneity, physical barriers, and the uplift of the Yungui Plateau were found to deeply affect the phylogeographic pattern and evolutionary history of *Cephalotaxus oliveri*, a perennial conifer endemic to China. In this study, we explored the phylogeography using three chloroplast sequences (*trn*L–*trn*F, *trn*T–*trn*D and *atp*B–*rbc*L) in 22 natural populations of *C. oliveri* distributed throughout its range. The Yungui Plateau populations of *C. oliveri* were revealed to origin ca. 9.15 Ma by molecular clock estimation, which is consistent with rapid uplift of the Qinghai–Tibetan Plateau (QTP) ca. 8–10 Ma. Additionally, geological effects of the Yungui Plateau were suggested to promote the rapid intra-specific differentiation of *C. oliveri* in the Pliocene and Early Pleistocene. The relatively low level of genetic diversity ($h = 0.719$, $\theta = 1.17 \times 10^{-3}$) and high population differentiation ($N_{ST} = 0.771$ and $G_{ST} = 0.642$) implied restricted gene flow among populations, which was confirmed by the Nested Clade Analysis (NCA). Mismatch distribution and haplotypes network provided evidences of recent demographic population expansion. Furthermore, the statistical dispersal–vicariance analysis indicated that the center of origin was in Central China. The comparison of haplotype distribution patterns indicated that the regions of HNHPS and HBLD were the potential refugia during the Pleistocene ice ages. Our results highlighted that habitat heterogeneity and physical barriers presenting in a species range can predict genetic patterns.

1. Introduction

Habitat heterogeneity and physical barriers play predominant roles in promoting population divergence and genetic structure. Different habitats perform different selection regimes, which constrain ecological and evolutionary trajectories of species (Southwood, 1977, 1988). Important characteristics of the environment which influence the selection regime include the variability of the spatial arrangement and temporal persistence within habitat patches (Marten et al., 2006; Gyllenberg and Hanski, 1992). The spatial and temporal settings of a habitat, then, strongly affect the evolution of species dispersal propensity, which affect not only its distributional range and divergence but also population genetics (Ribera and Vogler, 2000; Ribera et al., 2001). Turesson (1925) has indicated that habitat heterogeneity could have a profound effect as a selection pressure on the population genetic differentiation because individuals in different populations are tied to their particular environment. This may act as effective barriers promoting ecological divergence by local adaptation. Additionally, physical barrier is also expected to deeply affect the genetic structure of populations (Smith, 1999), since the barriers will promote fine-scale ecological variation and ultimately represent separated regions of unsuitable ecological conditions for a species (Lomolino et al., 2006). These unfavorable conditions are referred to extrinsic elements that determine the range limits of a species such as temperature and rainfall (Pyron and Burbrink, 2010). Hence, physical barrier will impede dispersal and limit range distribution between both sides (Kirkpatrick and Barton, 1997; Lomolino et al., 2006). With sufficient time, the genetic changes

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that accumulate in one population will be different from those in other populations isolated by physical barrier, resulting in genetic divergence. Such historical events can be revealed by examining contemporary genetic composition, which is likely accompanied by reduced within-population genetic variation and elevated among-population genetic differentiation (Schaal and Olsen, 2000).

The Yungui Plateau is situated in the southwestern China with an area of 3×10^5 km^2 at an elevation of about 1000–3000 m above sea-level. The terrain of the plateau is higher in its northwest than in southeast, and is divided into Yunnan Plateau and Guizhou Plateau by Mountain (Mt.) Wumeng. The western part of the Yungui Plateau mainly locates in Yunnan Province, whereas the eastern part in Guizhou Province. The Yungui Plateau represents one of the world's biodiversity hotspots. It has been shown to play an important role in revealing biological consequences of the Late Cenozoic orogenic events (Myers et al., 2000; Cheng et al., 2001; Liu et al., 2006; Li et al., 2012). Another reason that makes the geological effect of the Yungui Plateau crucial for species evolution is its close relationship with the Qinghai–Tibetan Plateau (QTP). The uplift of QTP has triggered the orogenic events of the Yungui Plateau. Since the Cenozoic, the Indian plate has kept moving northward. As a result, the movement of the QTP is violent, which induced the continuing uplift of the Yungui Plateau, contributing to the formation of its unique geomorphological configuration and complex land conditions (Wu et al., 2008). This significant increase in geological and ecological variables has promoted rapid divergence in small and isolated populations such as *Ligularia–Cremanthodium–Parasenecio* complex and *Babina pleuraden* (Liu et al., 2006; Li et al., 2012). Hence, it is of great interest to deduce whether the geological events of the Yungui Plateau is the main driving force for shaping the current genetic structure and biodiversity of plants in this region.

Cephalotaxus oliveri Masters is a perennial, coniferous shrub or small tree belonging to the family Cephalotaxaceae. The plant is up to 4 m in height, characterized by leafy branchlets oblong-elliptic in outline, with pollen-cone capitula axillary borne on lower side and toward distal end of the terminal branchlets (Fu et al., 1999). As an endemic species to China, *C. oliveri* is mainly dispersed in montane regions of the Yungui Plateau (Guizhou, southern and western Sichuan, eastern Yunnan), Central China (western Hubei, Hunan), East China (eastern Jiangxi) and South China (northern Guangdong), occurring primarily in humid and shady locations with an elevation between 300 and 1800 m (Fu et al., 1999). Its distributional areas always accompany with multiple geographical barriers. Most remarkably, the Mountains (Mts.) Daba, Wu and Xuefeng are located on the northeast boundary of the Yungui Plateau as a mountain system and separated the Yungui Plateau populations of *C. oliveri* from its neighboring populations. Another noticeable focus is the Mt. Wumeng, which is one of the highest mountains among the same latitudinal belt in the northern hemisphere. It restricts individual immigration of many species between the Yunnan Plateau and the Guizhou Plateau. The occurrence of these two physical barriers has recently been reported to affect the genetic structure of such species as *Eurycorymbus cavaleriei*, *Saruma henryi*, *Ligularia tongolensis*, *Terminalia franchetii*, and *Nouelia insignis* (Wang et al., 2009; Zhou et al., 2010; Wang et al., 2011a; Zhang et al., 2011; Gong et al., 2011).

In general, habitat heterogeneity has been defined concerning the number, extent (percentage cover, height/volume, patch size) and variety of physically structuring elements within a given ecological environment (Cordes et al., 2010). *C. oliveri* populations exhibit a range of diverse habitats. Firstly, its populations scatter in either broadleaf forest or conifer forest, where the plant community types are varied (Lang et al., 2011). Secondly, its western populations grow primarily in limestone mountainous region with an elevation between 1500 and 2700 m, where the rock is completely bare and the soil is shallow. The eastern populations, on the contrary, mainly disperse in sand shale hilly area with relative soft and fertile soil in an elevation below 1500 m. (Zhou et al., 1997; Chen et al., 2011b). Thirdly, temperature, rainfall, and light also exhibit significant heterogeneity, which mainly restrict the species distribution of *C. oliveri* (Ai et al., 2010). The temperature of the Yungui Plateau is lower and the rainfall is heavier than that of other regions (Wang et al., 2011b). Furthermore, the temperature and rainfall of the Yunnan Plateau is much higher than that of the Guizhou Plateau in winter but lower in summer (Wang et al., 2011b). For instance, the climatic characteristics of Sichuan and Chongqing are frequently cloudy, foggy and less sunny, resulting in the temperature being low whereas the rainfall heavy (Yi et al., 2007; Hu, 1964). Jiangxi, in contrast, has relatively high temperature and humidity, also with abundant rainfall (Gao, 1991). Finally, the populations of *C. oliveri* are naturally restricted to small and isolated areas separated by high mountains and deep valleys, which are largely congruent with the ecological characteristics of "old rare species" (Becker et al., 2011). Taken together, *C. oliveri* provides an ideal opportunity to examine the effects of habitat heterogeneity on the population genetic pattern in conifers.

The Chloroplast DNA (cpDNA) intergenic spacers have been used frequently to survey population variation and phylogeography of plants (Cannon and Manos, 2003; Honjo et al., 2004; Ikeda and Setoguchi, 2007; Chen et al., 2008). Their nearly neutral and uniparental inheritance, which allows the preservation of haplotypes over generations, are well suitable for locating refugia and postglacial recolonization routes (Petit et al., 2003; Heuertz et al., 2004; Grivet and Petit, 2003; Lascoux et al., 2003). Moreover, cpDNA markers are also powerful tools for inferring the population history, for example, population expansion or decline (Depaulis et al., 2005). Here, we surveyed sequence variations of chloroplast *trn*L–*trn*F, *trn*T–*trn*D, and *atp*B–*rbc*L within and between populations of *C. oliveri*, which *contributed to* a comprehensive understanding of its phylogeography. We mainly focused on the following issues: (1) to determine if the genetic structure of *C. oliveri* is related to its habitat heterogeneity and physical barriers, (2) to infer the impact of the Yungui Plateau uplift on the evolutionary history of *C. oliveri*, and (3) to locate its ice age refugia and reconstruct the biogeographic history.

2. Materials and methods

2.1. Sampling

In this study, we investigated the genetic variation and population structure in *C. oliveri*, which is endemic to China. In all, 22 natural populations distributed throughout its range were sampled across seven provinces and one city, including Hunan, Hubei, Jiangxi, Guangdong, Sichuan, Chongqing, Guizhou and Yunnan, (Fig. 1, Table 1). Among them, the populations of Yunan, Guizhou, and Sichuan were sampled from the Yungui Plateau. Fifteen individuals were collected from the populations, except GDDXS, JXYF, and JXXS, which only eleven, eight, and five surviving plants were found, representatively. In addition, one population of *C. fortunei* with 10 individuals was selected as outgroup. Young and healthy leaves were randomly sampled from individuals at least 10 m apart and immediately preserved in silica gel. All samples were stored at −20 °C until processed.

2.2. DNA extraction and sequencing

Total genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Su et al., 1998) and used as template in the polymerase chain reaction (PCR). Prelimin-