Molecular Phylogenetics and Evolution 59 (2011) 412–424

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Glacial survival east and west of the 'Mekong–Salween Divide' in the Himalaya–Hengduan Mountains region as revealed by AFLPs and cpDNA sequence variation in *Sinopodophyllum hexandrum* (Berberidaceae)

Yong Li [a,d], Sheng-Nan Zhai [b], Ying-Xiong Qiu [b,*], Yan-Ping Guo [c], Xue-Jun Ge [a,*], Hans Peter Comes [e]

[a] *Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China*
[b] *Key Laboratory of Conservation Biology for Endangered Wildlife of the Ministry of Education, College of Life Sciences, zhejiang university, Hangzhou 310058, China*
[c] *Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, and College of Life Sciences, Beijing Normal University, Beijing 100875, China*
[d] *School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China*
[e] *Department of Organismic Biology, Salzburg University, A-5020 Salzburg, Austria*

ARTICLE INFO

Article history:
Received 16 September 2010
Revised 19 November 2010
Accepted 21 January 2011
Available online 4 February 2011

Keywords:
AFLP
Chloroplast DNA
Glacial refugia
Himalaya–Hengduan Mountains
Molecular phylogeography
Sinopodophyllum hexandrum

ABSTRACT

Molecular phylogeographic studies have recently begun to elucidate how plant species from the Qinghai-Tibetan Plateau (QTP) and adjacent regions responded to the Quaternary climatic oscillations. In this regard, however, far less attention has been paid to the southern and south-eastern declivities of the QTP, i.e. the Himalaya–Hengduan Mountains (HHM) region. Here, we report a survey of amplified fragment length polymorphisms (AFLPs) and chloroplast DNA (cpDNA) sequence variation in the HHM endemic *Sinopodophyllum hexandrum*, a highly selfing alpine perennial herb with mainly gravity-dispersed berries (105 individuals, 19 localities). We specifically aimed to test a vicariant evolutionary hypothesis across the 'Mekong–Salween Divide', a known biogeographic and phytogeographic boundary of north-to-south trending river valleys separating the East Himalayas and Hengduan Mts. Both cpDNA and AFLPs identified two divergent phylogroups largely congruent with these mountain ranges. There was no genetic depauperation in the more strongly glaciated East Himalayas (AFLPs: $H_E = 0.031$; cpDNA: $h_S = 0.133$) compared to the mainly ice-free Hengduan Mts. (AFLPs: $H_E = 0.037$; cpDNA: $h_S = 0.082$), while population differentiation was consistently higher in the former region (AFLPs: $\Phi_{ST} = 0.522$ vs. 0.312; cpDNA: $\Phi_{ST} = 0.785$ vs. 0.417). Our results suggest that East Himalayan and Hengduan populations of *S. hexandrum* were once fragmented, persisted *in situ* during glacials in both areas, and have not merged again, except for a major instance of inter-lineage chloroplast capture identified at the MSD boundary. Our coalescent time estimate for all cpDNA haplotypes (c. 0.37–0.48 mya), together with paleogeological evidence, strongly rejects paleo-drainage formation as a mechanism underlying allopatric fragmentation, whereas mountain glaciers following the ridges of the MSD during glacials (and possible interglacials) could have been responsible. This study thus indicates an important role for mountain glaciers in driving (incipient) allopatric speciation across the MSD in the HHM region by causing vicariant lineage divergence and acting as barriers to post-divergence gene flow.

1. Introduction

The Qinghai-Tibetan Plateau (QTP) in the western part of China forms a rather specialized area chiefly on account of its great elevation [4500 m above sea level (a.s.l.) on average] and size (2.5×10^6 km^2; Zhou et al., 2006). The Himalayas (reaching 8848 m a.s.l.) form the southern frontier of this highest plateau in the world, whereas the Hengduan Mts. of Southwest China ranges its south-eastern margin. When compared to the QTP proper, plant species diversity and endemism is notably higher in the Himalaya–Hengduan Mountains (HHM) region, which comprises major parts of three globally recognized 'biodiversity hotspots', i.e. 'Himalaya', 'Indo-Burma', and 'Mountains of Southwest China' (Myers et al., 2000; Conservation International, 2005; see also Wu, 1988; Li and Li, 1993).

In recent years, a growing body of molecular phylogeographic studies has begun to elucidate how plant species occurring in the QTP and adjacent regions responded to the Quaternary climatic oscillations (<2.4–1.8 million years ago, mya) by using DNA sequence variation from mainly the chloroplast (cp) and/or mitochondrial (mt) genomes (reviewed in Qiu et al., 2011). For a number of coniferous forest tree species and (sub)alpine herbs and shrubs, presently occurring on the QTP platform, there is

---

* Corresponding authors. Fax: +86 571 86432273 (Y.-X Qiu).
E-mail addresses: qyxhero@zju.edu.cn (Y.-X. Qiu), xjge@scbg.ac.cn (X.-J. Ge).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.01.009

now firm molecular evidence that they re-colonized this area from glacial refugia located at lower elevations at the (north-)eastern and south-eastern plateau edges, the latter including the mostly ice-free Hengduan Mts. (*Juniperus przewalskii*: Zhang et al., 2005; *Picea crassifolia*: Meng et al., 2007; *Metagentiana striata*: Chen et al., 2008a,b; *Pedicularis longiflora*: Yang et al., 2008). By contrast, in several other instances, molecular data were consistent with hypotheses of glacial *in situ* survival on the QTP platform itself (*Aconitum gymnandrum*: Wang et al., 2009a; *Potentilla fruticosa*: Li et al., 2010; Shimono et al., 2010; *P. glabra*: Wang et al., 2009b; *Juniperus* spp.: Opgenoorth et al., 2010; *Hippophae tibetana*: Wang et al., 2010a), which seems plausible as there was never a unified ice sheet during the Late Quaternary (Shi, 2002; Owen et al., 2008). Recently, there has also been an increased appreciation of differences in topography in explaining different demographic histories of QTP plants during the Quaternary (Li et al., 2011; Opgenoorth et al., 2010).

Despite its biotic richness and high number of endemic plant species, far less attention has been paid to the HHM region in phylogeographic studies (but see Gao et al., 2007; Yang et al., 2008; Cun and Wang, 2010; Opgenoorth et al., 2010). This mountain belt is uniformly characterized by vertical distributions of forest to subglacial ecosystems, starting from dry, riparian vegetation up to and dominated by sub-alpine coniferous (e.g., *Tsuga*, *Picea*. *Abies*, *Larix*, *Juniperus*) forest resembling mountain taiga (Wu, 1998; Wu and Wu, 1998). However, when compared to the Hengduan Mts., the eastern Himalayan region is impoverished from east to west in plant species diversity as well as endemism, in both genera and families (Li, 1996; Wu, 1998; Wu and Wu, 1998; Mutke and Barthlott, 2005).

It has been hypothesized (Opgenoorth et al., 2010) that the large altitudinal amplitudes of the HHM region, together with their more varied topographies, may have allowed plant populations, such as those of *Juniperus*, to survive glacial periods *in situ* by tracking suitable climatic conditions (or niches) over much shorter distances than those prevailing in the north-eastern parts of the QTP. On the other hand, this hypothesis of persistence could not be supported by a recent phylogeographic study using cp/mtDNA and nuclear DNA from the *LEAFY* gene to analyse the genetic structure of *Tsuga dumosa* (Cun and Wang, 2010). Rather, in this sub-alpine conifer, the distribution of cytoplasmic haplotypes, together with fossil evidence, indicated (re-)colonization of the eastern Himalayas from the Hengduan Mts., possibly during the mid-Pleistocene (c. 0.81 mya). Also in the same region, a similar range expansion, probably during the last interglacial, was inferred for the alpine herb Pedicularis. *longiflora* (Yang et al., 2008). Again, it appears that broadly co-distributed (sub-)alpine conifers and herbs in the HHM region exhibit different demographic histories in terms of long-term glacial *in situ* survival vs. (temporally discordant) east-to-west expansion. Notably, for *Taxus wallichiana*, a conifer of mixed/broad-leaved montane forests, Gao et al. (2007) reported a major phylogeographic break associated with the steep mountains and deep valleys of the Mekong (Lancang) and Salween (Nujiang) rivers. This 'Mekong–Salween Divide' (MSD), which runs from North to South with over 4000 m (a.s.l.) altitudes, is known as a biogeographic barrier (Kingdon Ward, 1921), and separates two Chinese floristic provinces, i.e. the 'Eastern Himalaya North Wing' in the West and the 'Hengduan Mountain Region' in the East (Wu, 1998; Wu and Wu, 1998; see Fig. 1A and B). The large watersheds of the Mekong and Salween, or other topological effects, may have prevented species from spreading, and/or promoted population differentiation and (incipient) allopatric speciation by acting as barriers to gene flow, but evidence for this is so far very limited in both plants and animals (Zhang et al., 2006; Gao et al., 2007; Thinh et al., 2010).

In order to further test the above predictions of glacial persistence, westward range expansion, and/or vicariant segregation across the MSD, we here aim to recover the phylogeographic history of a rhizomatous perennial herb endemic to the HHM region, *Sinopodophyllum hexandrum* (Royle) Ying (Berberidaceae/Podophylloideae), commonly known as the Himalayan Mayapple. Recent phylogenetic and morphological studies (Wang et al., 2009c) confirmed *S. hexandrum* as sister to the eastern North American *Podophyllum peltatum* L., from which it mainly differs by pollen released in tetrads rather monads (Ying, 1979), a self-compatibility system (which is atypical for Podophylloideae in general; Shaw, 2002, 2009), and an unusual auto-pollination mechanism (Xu et al., 1997; see below).

Populations of *S. hexandrum* are scattered in isolated stands throughout the HHM region, from eastern Afghanistan to Southwest China (northern Yunnan Province), extending northwards into the western Chinese provinces of Sichuan, Shanxi, and Gansu (Chatterjee, 1952; Ying, 1979; Fu, 1992; Shaw, 2000, 2002; see Fig. 1A). Rare occurrences are also known from the central parts of the QTP (see dot map given by Shaw, 2000). Typical habitats are open mountain slopes and shady coniferous or *Quercus* forests, *Juniperus-Rhododendron* scrub, or occasionally alpine pastures in moist places, with an altitude range of c. (1800–) 2700–4500 m a.s.l. (Ma and Hu, 1996; Shaw, 2000, 2002). Although this widespread species is known for an unusual degree of morphological variation (e.g., in foliar characters, flower position, flower color, anther length), Shaw (2002, p. 380) concluded that assignment of most specimens to a formal variety would be "rather arbitrary". Population sizes of *S. hexandrum* are generally small, ranging from 40 to 700 individuals per location (Bhadula et al., 1996). Increasing demand for *S. hexandrum* rhizome for medicinal use and more recently for the semisynthesis of anti-cancer drugs (Van Uden et al., 1989; Canel et al., 2000) has led to over collection from the wild over the last century (Shaw, 2002). This has resulted in a once common HHM plant being now treated as 'endangered' or 'threatened' in India and China (Gupta and Sethi, 1983; Wang and Xie, 2004) due to anthropogenic overexploitation and a low rate of natural regeneration (Airi et al., 1997; Bhadula et al., 1996; Ma and Hu, 1996; Nadeem et al., 2000; Shaw, 2002).

So far, there have been no studies based on DNA sequence variation in *S. hexandrum*. Most studies have focussed on local population genetic structure in western China (Sichuan) or the southern Himalaya (north India) using allelic variation at allozymes (e.g., Bhadula et al., 1996), inter-simple sequence repeats (ISSRs) (e.g., Xiao et al., 2006a; Alam et al., 2008), random amplified polymorphic DNAs (RAPDs) (e.g., Alam et al., 2009), or amplified fragment length polymorphisms (AFLPs) (e.g., Xiao et al., 2006b). While these studies generally revealed low levels of within-population genetic diversity and high levels of among-population genetic differentiation, they were inherently limited by not covering a broader regional scale, especially encompassing the supposedly important MSD barrier. Moreover, the joint use of molecular markers derived from different genomes provides a more complete description of population structure and insights into population history and dynamics, particularly for comparisons of maternally inherited organelle and bi-parentally inherited nuclear markers (Schaal et al., 1998; Petit et al., 2005; Guan et al., 2010). Here we report the results of a phylogeographic study on *S. hexandrum* based on cpDNA and AFLP variation surveyed in the HHM region of western China (Fig. 1B).

We focus on the following questions: (i) How is genetic diversity distributed within and among populations of the self-compatible and auto-pollinating *S. hexandrum*? (ii) Are populations in the East Himalayas genetically depauperate with respect to those in the Hengduan Mts., and/or do the former exhibit signs of range expansion, as would be expected in case of a westward spread from a Hengduan glacial refuge as inferred for both *P. longiflora* (Yang et al., 2008) and *T. dumosa* (Cun and Wang, 2010)? (iii) Or