Molecular Phylogenetics and Evolution 93 (2015) 63–76

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Mitochondrial introgression and complex biogeographic history of the genus *Picea* ☆

Jin-Hua Ran [1], Ting-Ting Shen [1], Wen-Juan Liu, Pei-Pei Wang, Xiao-Quan Wang *

State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

ARTICLE INFO

Article history:
Received 15 March 2015
Revised 18 July 2015
Accepted 25 July 2015
Available online 30 July 2015

Keywords:
Biogeography
Molecular phylogeny
Bering Land Bridge
Mitochondrial DNA introgression
Divergence times
Picea

ABSTRACT

Biogeographic history of plants is much more complex in the Northern Hemisphere than in the Southern Hemisphere due to that both the Bering and the North Atlantic land bridges contributed to floristic exchanges in the Cenozoic, which led to hybridization between congeneric species from different continents. It would be interesting to know how intercontinental gene flow and introgression have affected plant phylogenetic reconstruction and biogeographic inference. In this study, we reinvestigated the phylogenetic and biogeographic history of *Picea*, a main component of the Northern Hemisphere forest with many species that originated from recent radiation, using two chloroplast (cp), one mitochondrial (mt) and three single-copy nuclear gene markers. The generated gene trees are topologically highly discordant and the geographically closely related species generally show a close affinity of mtDNA rather than cp- or nuclear DNA, suggesting that inter- and intra-continental gene flow and mtDNA introgression might have occurred commonly. However, all gene trees resolved *Picea breweriana* as the basal-most lineage, which, together with fossil evidence, supports the North American origin hypothesis for the genus. Both dispersal and vicariance have played important roles in the evolution of *Picea*, and the Bering Land Bridge could have mediated the "North America to Eurasia" dispersal at least two times during the Miocene and Pliocene. Our study again demonstrates the importance of applying data from three genomes for a clear understanding of evolutionary histories in the pine family. Any markers from a single genome alone will not reveal a clear picture of the phylogenetic relationships among closely related congeneric species. In particular, mtDNA markers should be cautiously used, considering that introgression of the maternally inherited mtDNA with a lower rate of gene flow (by seeds) could have occurred much more frequently than that of the paternally inherited cpDNA with a higher rate of gene flow (by pollen) in Pinaceae.

1. Introduction

The frequent climatic oscillations and environmental changes during and after the late Tertiary, especially the recurrence of the Quaternary glacial–interglacial cycles, have played important roles in shaping the present distribution and diversity of plants in the Northern Hemisphere (Hewitt, 1996). Generally, allopatric fragmentation happened and many species retreated to isolated refugia during the glacial periods. In contrast, divergent lineages or species isolated in different refugia with incomplete reproductive barriers might have expanded their distributions, and sometimes became tangled by secondary contact during the interglacial or postglacial periods (Jaramillo-Correa et al., 2004, 2006; Anderson et al., 2006; Du et al., 2009; Li et al., 2010; Sun et al., 2014). In addition, due to the sea level fluctuations in response to the late Tertiary and Quaternary climatic changes (Graham, 1993, 1999; Manchester, 1999; Wen, 1999; Milne, 2006), the Bering and the North Atlantic land bridges (BLB and NALB) which connected between North America and Eurasia contributed to floristic exchanges in the Northern Hemisphere (Hopkins, 1967; McKenna, 1983; Tiffney, 1985a; Milne, 2006). Previous studies suggested that most Asian-North American disjunct lineages diverged in the Miocene and the BLB had acted as an important corridor for the dispersal of temperate disjunct lineages (see review by Wen et al., 2010). It is particularly interesting that, for the forest components, most angiosperm genera might have originated in eastern Asia and subsequently dispersed to North America whereas most conifer genera possibly originated in North America and then migrated to Asia (Donoghue and Smith, 2004; Wen et al., 2010; Wang and Ran, 2014). Notably, many plant genera could have experienced more than one dispersal and (or) vicariance

---

☆ This paper was edited by the Associate Editor Stefanie M. Ickert-Bond.

* Corresponding author.

E-mail address: xiaoq_wang@ibcas.ac.cn (X.-Q. Wang).

[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.ympev.2015.07.020
1055-7903/

events in their evolutionary histories, such as *Pinus* (Eckert and Hall, 2006) and *Abies* (Semerikova and Semerikov, 2014a, 2014b). Furthermore, long distance dispersal (LDD) could also have contributed to the complexity of the Northern Hemisphere floras (Hagen et al., 2001; Abbott and Brochmann, 2003; Alsos et al., 2007; Bendiksby et al., 2014; Zhang et al., 2014; Xiang et al., 2015). Therefore, the biogeographic history of some Northern Hemisphere plant groups is very complicated and remains unresolved.

Recently, an increasing number of studies have used DNA markers from different genomes to reconstruct the evolutionary and biogeographic history for plant groups (Mason-Gamer, 2001; Kim et al., 2008; Peng and Wang, 2008; Guo et al., 2012; Yang et al., 2012; Zhang et al., 2012). Excluding stochastic and systematic errors, the phylogenetic conflicts among DNA markers from different genomes were always attributed to interspecific hybridization, introgression, incomplete lineage sorting, gene paralogy and horizontal gene transfer (Maddison, 1997; Mayol and Rosselló, 2001; Bergthorsson et al., 2003; Linder and Rieseberg, 2004; Baum, 2007; Richardson and Palmer, 2007; Peng and Wang, 2008; Zou and Ge, 2008; Zhang et al., 2012). Horizontal gene transfer and gene paralogy are relatively easy to be resolved because the former was found mostly in mitochondrial DNA (Bergthorsson et al., 2003; Richardson and Palmer, 2007), and the latter could be tested by using single-copy genes (Zhang et al., 2012). In contrast, hybridization, introgression, or incomplete lineage sorting are difficult to discriminate but are responsible for most instances of phylogenetic conflicts among genes.

The rates of introgression are negatively correlated to the rates of gene flow (Du et al., 2009; Petit and Excoffier, 2009). In plants, except those with long-distance dispersed seeds/fruits by wind or birds, seed dispersal is often considerably less effective than pollen dispersal (Petit et al., 1993, 2005). When the dispersal ability of seed is particularly lower than that of pollen, variation patterns of maternally inherited DNA are often more influenced by geography than by taxonomy in some plants, such as wind-pollinated plants (Wolf et al., 1997; Dumolin-Lapegue et al., 1999; McKinnon et al., 2001; Petit and Excoffier, 2009). Mitochondrial (mt) and chloroplast (cp) DNAs show very different levels of gene flow in Pinaceae due to their respective maternal and paternal models of transmission, and wind pollination (Neale and Sederoff, 1988; Gillham, 1994; Mogensen, 1996). Therefore, in the pine family, the use of both cp- and mt-DNA, combined with biparentally inherited nuclear genes, can provide comparable and genetically unlinked markers for us to disentangle the complex evolutionary and biogeographic processes, to discriminate among effects of hybridization, introgression and incomplete lineage sorting that could lead to topological incongruence among gene trees, and to evaluate the directionality of introgression and gene flow.

Picea (spruce), comprising around 34 species, is the third largest genus in Pinaceae with a wide distribution in the Northern Hemisphere. Our previous study has revealed a complex evolutionary and biogeographic history of the genus (Ran et al., 2006). Cross experiments showed hybridization between many geographically isolated spruce species, even from different continents, such as *Picea mariana*, *Picea rubens* (North America) and *Picea omorika* (Europe) (Mikkola, 1969; Ledig et al., 2004). The historical interspecific hybridization and reticulate evolution have led to the difficulties in the phylogenetic and biogeographic studies of this genus. Our previous study based on combined two cpDNA fragments (*trn*C-*trn*D and *trn*T-*trn*F) suggested that *Picea* originated from North America, then dispersed to Eurasia by the BLB (Ran et al., 2006). However, two recent molecular studies argued that *Picea* originated from Eurasia (Bouillé et al., 2011; Lockwood et al., 2013), although the cpDNA phylogeny reconstructed by Bouillé et al. (2011) is topologically very similar to that reported in our study. Also, the mtDNA phylogeny reconstructed by Bouillé et al. (2011) has a low resolution, and did not resolve the basal group of the genus. Notably, Lockwood et al. (2013) combined all studied sequences from cp- and mt-DNA as well as the low-copy nuclear gene *4CL* for phylogenetic analysis, but they did not conduct any test for the congruence among the datasets, although Bouillé et al. (2011) found that phylogenies of the genus reconstructed from cp- and mt-DNA are highly incongruent. Additionally, due to radiative speciation and morphological convergence, the phylogenetic positions of many species of *Picea* remain unresolved (Ran et al., 2006; Bouillé et al., 2011; Lockwood et al., 2013). Moreover, recent phylogeographic studies showed that many spruce species experienced hybridization and mitochondrial introgression (Bouillé and Bousquet, 2005; Jaramillo-Correa and Bousquet, 2005; Pelgas et al., 2006; Du et al., 2009, 2011; Gérardi et al., 2010; Li et al., 2010; Zou et al., 2013; Sun et al., 2014). Therefore, a comprehensive study is needed to reinvestigate the origin, evolution and biogeography of the genus.

In this study, we reconstruct the phylogeny of *Picea* by using two paternal chloroplast, one maternal mitochondrial, and three single-copy nuclear DNA markers, respectively. By comparing the topologies of different gene trees, together with molecular dating and ancestral area reconstruction, we not only reinvestigate the evolutionary and biogeographic history of the genus, but also discuss the influences of hybridization and introgression on phylogenetic reconstruction and biogeographic inferences, which were neglected in previous studies. Our study may also shed light on biogeographic studies of plants in the Northern Hemisphere.

2. Materials and methods

2.1. Taxon sampling

We sampled a total of 86 individuals representing all 34 spruce species recognized by Farjón (1990), with the exception of *Picea aurantiaca*, a name that was not accepted by *Flora of China* (Fu et al., 1999). Most species were represented by more than one individuals. *Cathaya argyrophylla*, *Pinus thunbergii* and *Pinus strobus* were sampled as outgroups because of a close relationship between *Picea* and the two genera *Cathaya* and *Pinus* (Wang et al., 2000; Lin et al., 2010). The information of all samples is shown in Supplementary Table S1.

2.2. DNA extraction, PCR amplification, cloning and sequencing

Total DNAs were extracted from fresh or silica gel dried leaves using the modified CTAB method following Rogers and Bendich (1988). Two chloroplast (*trn*C-*trn*D and *trn*T-*trn*F) and one mitochondrial (*rps*3) DNA fragments as well as three single-copy nuclear genes (*FLOWERING LOCUS T/TERMINAL FLOWER 1*, *FTL*1; *LEAFY*, *LFY*; *STOMATAL DENSITY AND DISTRIBUTION 1*, *SDD*1) were amplified following the PCR protocol of Ran et al. (2006), except that a different annealing temperature was used for some of the markers. The *LFY* was proved to be a single-copy gene in gymnosperms by southern blotting (Yang et al., 2012). The homologs of *FTL*1 and *SDD*1 from the genome databases of three conifers (*Picea abies*, *P. glauca*, and *P. taeda*) and some angiosperm species were compared, and the orthologs of the two genes were finally selected. The amplification and sequencing primers are shown in Supplementary Table S2, of which all primers of the three nuclear genes were newly designed. After purification with the TIANgel Midi Purification Kit (Tiangen), the PCR products of the cp- and mt-DNA fragments were sequenced directly, whereas those of the nuclear genes were cloned with the pEASY-T3 Cloning Kit