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Population genetic structure and interspecific differentiation between *Acer davidii* Franchi. and *A. morrisonense* Hayata (Aceraceae) based on SSR markers

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ABSTRACT

Acer davidii Franch. and *A. morrisonense* Hayata are two important forest tree species (Aceraceae) endemic to mainland China and the Taiwan area, respectively. To investigate population structure and interspecific differentiation between them, we characterized a set of novel microsatellite markers using Illumina sequencing technology. The cross-species amplification analysis showed that 11 out of 21 polymorphic SSR primers of *A. davidii* also exhibited polymorphisms in *A. morrisonense*. At the species level, *A. davidii* has a slightly higher genetic diversity (mean observed heterozygosity, H_O = 0.180) than *A. morrisonense* (H_O = 0.119). AMOVA showed that most of the variations in *A. davidii* occurred among individuals within populations and within individuals. Bayesian clustering analyses demonstrated that the two maple species formed two clear genetic lineages. PCoA showed that *A. davidii* and *A. morrisonense* were significantly divided into two genetic groups. In addition, asymmetrical weaker gene flow was detected among the two forest tree species. These results suggest that the long term geographic isolation between the mainland and Taiwan may have resulted in a high level of genetic differentiation between these two important maple species.

1. Introduction

Acer L. (Aceraceae) is an important tree genus in forest eco-systems in the Northern Hemisphere. This genus contains approximately 129 species with many infraspecific taxa. The maple species are mainly trees or shrubs, occupying a significant part of the temperate regions of East Asia, eastern North America and Europe (van Gelderen et al., 1994; Wu and Peter, 2012). In China, 99 species have been reported, 61 of which are endemic (Xu et al., 2008). Thus, China is considered to host the greatest diversity of the genus *Acer* (Suh et al., 2000). In the Northern Hemisphere, the *Acer* species is commonly used in commercial products, such as furniture and lumber (Yaltirik, 1970). Meanwhile, maple's roots have also been used in traditional medicine in East Asia (e.g., China, Japan, and Korea) and North America (Bi et al., 2016).

A. davidii Franch. and *A. morrisonense* Hayata are two important forest species in the genus *Acer* of the family Aceraceae (Wu and Peter, 2012). They are both dominant and constructive tree species in the local forest community in East Asia. *A.*

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davidii, often occurring in thin forests at an altitude of 500–1500 m, is endemic to central and southwestern China. *A. morrisonense* is restricted to the central mountains of Taiwan and generally occurs in marginal forests at an altitude of 1800–2300 m. The two maple species have important ecological and economical values and play key roles in local forest ecosystems due to their strong resistance to cold and drought (Li and Lo, 1993; Wu and Peter, 2012; Han et al., 2016). However, prior to this study, DNA molecular marker data for the two maple species were unavailable, which seriously hindered the study of population genetics and species differentiation.

In recent years, with the development of modern molecular biology technology, polymorphic molecular markers, isolated based on next generation sequencing, have been largely used to study population genetic diversity and the species evolution of organisms (Hadrys et al., 1992; Thomson et al., 2010). Microsatellites, also known as simple sequence repeats (SSRs), have been widely developed and used for population-level studies in the fields of genetics, ecology, evolution, conservation and management (Morgante et al., 2002; György et al., 2014). These studies have profoundly revealed population genetic structure and the interspecific divergence of some endangered species (Ellegren, 2004; Yıldırım et al., 2014). For example, important conservation and management strategies were created for an endangered species, *Acer yangbiense*, based on the analysis of population genetic structure of polymorphic microsatellite markers (Yang et al., 2015). Meanwhile, moderate levels of genetic diversity and population differentiation were determined for an endangered medicinal plant, *Phellodendron amurense*, inferred from the genetic variations of eight nuclear SSR primers (Yang et al., 2016). In addition, the significant genetic divergence in the Changbai mountain areas of *Taxus cuspidate* was revealed by SSR markers (Cheng et al., 2015).

In the present study, we firstly developed and characterized a set of novel microsatellite loci primers for two maple species by using Illumina paired-end sequencing, and we further used these polymorphic SSR markers to detect the genetic diversity and the pattern of species differentiation between *A. morrisonense* and *A. davidii*. To the best of our knowledge, this is the first development of a large number of polymorphic microsatellites for two maple species based on next generation sequencing technology. This knowledge of population genetics and species divergence will very helpful for further understanding the evolutionary history and differentiation mechanisms of forest tree species distributed throughout mainland China and Taiwan.

2. Material and methods

2.1. Microsatellite primer design, amplification and assessment

Illumina high-throughput sequencing of one individual of *A. davidii* was processed by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China), and the sequencing reaction was conducted on the HiSeq2000 platform with 125 bp pair-end reads. In total, 52.8 M clean reads were obtained and were then de novo assembled into 87,335 contigs using CLC Genomics Workbench v7.0 (CLC Bio, Aarhus, Denmark). The resultant contigs were screened for microsatellites using the software SciRoKo v3.4 (Kofler et al., 2007). Primer pairs were designed for 1025 microsatellite loci with suitable flanking regions using the program Primer Premier v5.0 (Premier Biosoft, Palo Alto, CA, USA). The criteria for primer design were as follows: (1) primer size from 18 to 25 bp; (2) primer melting temperate from 50 to 63 °C; (3) PCR product size from 100 to 300 bp; and (4) GC content from 40 to 60%.

A total of forty-two individuals from seven wild populations of *A. davidii*, and forty-three individuals from one wild population of *A. morrisonense* were sampled to detect the polymorphism of the microsatellite markers isolated (Table 1). Genomic DNA was extracted from silica-dried leaves using a modified CTAB method (Doyle, 1987). PCR amplifications were performed at a 10-μl volume including 5 μl of mixture (0.3 μM dNTPs, 0.8 U *Taq* DNA Polymerase), 0.3 μM each primer, ~10 ng

Table 1
Locations of sampled populations of *Acer davidii* and *A. morrisonense*.

Species	Population code	Location	*N*	Geographic coordinates	Altitude (m)
Acer davidii	D1	Guangtoushan, Shaanxi	4	108°46′46.87″ 33°51′11.558″	2800
	D2	Longmendong, Longxian, Shaanxi	3	106°39′47.302″ 35°04′12.109″	1500
	D3	Chenjiagou, Zhashuixian,Shaanxi	3	109°33′28.82″ 33°31′7.82″	1890
	D4	Beigou, Zhashuixian,Shaanxi	3	109°30′10.69″ 33°41′54.69″	1865
	D5	Xiaonangou, Zhashuixian, Shaanxi	4	108°52′6.65″ 33°47′21.32″	1872
	D6	Fengyukou, Shaanxi	5	108°49′22.68″ 33°49′13.95″	1540
	D7	Taibaishan,Shaanxi	20	107°42′19.847″ 34°05′21.254″	1133
A. morrisonense	M	Hehuanshan,Taiwan	43	121°17′12.605″ 24°09′41.932″	2200

Note: *N* = number of individuals.