

Resource Article: Genomes Explored

The genome of the Paleogene relic tree *Bretschneidera sinensis*: insights into trade-offs in gene family evolution, demographic history, and adaptive SNPs

Hai-Lin Liu^{1,2,3,4}, AJ Harris^{1,5}, Zheng-Feng Wang^{1,6,7,8*},
Hong-Feng Chen^{1,5*}, Zhi-An Li^{1,6,7,8}, and Xiao Wei⁹

¹Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China, ²University of Chinese Academy of Sciences, Beijing, 100049, China, ³Environmental Horticulture Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, China, ⁴Key Laboratory of Ornamental Plant Germplasm Innovation and Utilization, Guangzhou, 510640, China, ⁵Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China, ⁶Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, 511458, China, ⁷Center of Plant Ecology, Core Botanical Gardens, Chinese Academy of Sciences, Guangzhou, 510650, China, ⁸Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China, and ⁹Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, 541006, China

*To whom correspondence should be addressed. Email: wzf@scib.ac.cn (Z.-F.W.); h.f.chen@scib.ac.cn (H.-F.C.)

Received 10 November 2021; Editorial decision 12 January 2022

Abstract

Among relic species, genomic information may provide the key to inferring their long-term survival. Therefore, in this study, we investigated the genome of the Paleogene relic tree species, *Bretschneidera sinensis*, which is a rare endemic species within southeastern Asia. Specifically, we assembled a high-quality genome for *B. sinensis* using PacBio high-fidelity and high-throughput chromosome conformation capture reads and annotated it with long and short RNA sequencing reads. Using the genome, we then detected a trade-off between active and passive disease defences among the gene families. Gene families involved in salicylic acid and MAPK signalling pathways expanded as active defence mechanisms against disease, but families involved in terpene synthase activity as passive defences contracted. When inferring the long evolutionary history of *B. sinensis*, we detected population declines corresponding to historical climate change around the Eocene–Oligocene transition and to climatic fluctuations in the Quaternary. Additionally, based on this genome, we identified 388 single nucleotide polymorphisms (SNPs) that were likely under selection, and showed diverse functions in growth and stress responses. Among them, we further found 41 climate-associated SNPs. The genome of *B. sinensis* and the SNP dataset will be important resources for understanding extinction/diversification processes using comparative genomics in different lineages.

Key words: gene family, genome assembly, population genetics, resequencing, SNP

1. Introduction

Relic species are surviving members of lineages that were once widespread geographically and/or contained considerable taxonomic diversity that is lacking today. Although many relict species are currently confined to historical refugia and are endangered, they are remarkable for their persistence, whereas other related lineages or populations have become extinct. Understanding how relic species have survived historical environmental changes, including under recent anthropogenic influence, is fundamental to conservation and restoration studies.^{1–7} Considering many relic lineages contain only a few species or are monotypic,³ their genomes can provide species-specific information, such as on gene family evolution, demographic history, and adaptive single nucleotide polymorphisms (SNPs), to support long-term survival. With the rapid development of genome sequencing techniques, it is increasingly possible to obtain the whole genomes relatively easily, which benefits the understanding and conservation of relic species.

The relic species, *Bretschneidera sinensis* Hemsl ($2n = 18$; Fig. 1),⁸ is a relic deciduous broad-leaved forest tree that was once a component of the boreotropical flora found throughout the Northern Hemisphere.⁹ However, in modern times, *B. sinensis* is regionally endemic and mainly restricted to elevations of 300–1,700 m in remote mountainous areas of southern China at a latitude between 20°N and 30°N with other scattered individuals occurring in northern Vietnam, Thailand, and Myanmar. As with many relic plants, all natural population sizes of *B. sinensis* are small,¹⁰ often comprising 30 or less mature individuals in each population based on field investigations.¹¹ Presently, the species is listed as a Category-I endangered species in the ‘Key List of Protection of Wild Plants in China’ and as endangered globally by the International Union for Conservation of Nature Red List.^{12,13} Despite its small populations and concerning

conservation status, *B. sinensis* occupies a larger geographic breadth than many relic species, and, therefore, occurs in a relatively wide range of environments. This indicates that this species may have an evolutionary mechanism or adaptive genetic variation that supports its persistence.

Since *B. sinensis* was first discovered at the end of the 19th century, its taxonomic status has been controversial.^{10,14,15} Within various taxonomic treatments, it has been classified into different families and orders, including Brassicales and Sapindales. However, classification systems broadly agree that the *Bretschneidera* genus should be regarded as monotypic because no close living relatives of the species are known to exist. Moreover, treatments also agree that *B. sinensis* belongs within the malvids lineage, which includes both Brassicales and Sapindales.¹⁶ The phylogeny using the complete chloroplast genome sequence of *B. sinensis* and other species of malvids further indicated that *B. sinensis* is genetically closest to *Carica papaya*.¹⁷

In addition to taxonomy, other studies on *B. sinensis* have primarily focussed on its conservation, especially to identify the factors that possibly underlie its rarity and endangered status, such as seed germination,¹⁸ plant growth rates,^{19,20} water and light utilization,^{21,22} leaf and root anatomy,^{23–25} reproductive strategies,^{26,27} and genetic diversity.^{10,11} Results from these studies showed that, in the lab, the seed germination rate of *B. sinensis* was about 70%, but can be increased to 80% with gibberellic acid (GA3) treatment.¹⁸ However, the seed germination rate dramatically decreases to ~15% in the field.²⁰ With respect to growth rate, *B. sinensis* is a slow- to medium-growing plant exhibiting a curvilinear pattern of height gain; increasing in rate from the seedling to juvenile stage (~0.26 m/year) but decreasing in the second and third years (~0.18 m/year).^{19,20} The growth rate gradually increases again beyond the fourth year, but at only ~0.40 m/year, and remains lower than many other local tree



Figure 1. Representative photographs of *Bretschneidera sinensis*, including the whole tree, flowers, and fruits.