The endangered plant Tetracentron sinense Oliv. (Tetracentraceae) populations: genetic diversity, structure and dynamic history based on SSR markers for its conservation

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Research Article

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Abstract

Tetracentron sinense Oliver, as a tertiary living fossil, a dramatic decline in *T. sinense* population amounts, genetic resources depletion and recent human activities have shaped habitat fragmentation of relict and endangered plants, although there is ample evidence of its great medicinal, economic and ecological value. However, little is known about the genetic evolution of *T. sinense*. With this work, 193 individuals from 22 natural *T. sinense* populations regarding its genetic diversity, genetic differentiation, and demographic history using simple sequence repeat (SSR) markers to clarify its evolution models and develop scientific conservation strategies. We evaluated the genetic diversity, population structure and demographic history of 193 *T. sinense* individuals based on 14 SSR markers. At the species level, *PPL, I* and *He* were 100%, 1.631 and 0.559, respectively. At the population level, *Na*, *Ne*, I, *Ho* and He were 3.221, 2.505, 0.937, 0.434 and 0.566, respectively. The results revealed high genetic diversity at the species level and within populations. Individuals were structured into three main clusters ($K = 3$) with significant genetic differentiation ($Fst = 0.31$). Demographic history analysis showed that *T. sinense* differentiated according to the radial differentiation model. The differentiation occurred 1.115×10^4 to 2.23×10^4 years ago during Last Glacial Maximum. The twenty–two *T. sinense* populations revealed moderate genetic diversity and seemed to be structured into three clusters with high differentiation suggesting its preserved the evolutionary potential and the Hengduan Mountains and Qinling Mountains act as the two major glacial refuges. High differentiation caused by long–term geographic isolation may lead to the population extinction. The radial differentiation model suggested that *T. sinense* originated from common ancestor.

Introduction

Forest tree species are the foundation of ecosystems and make up a vast majority of the world's terrestrial biodiversity (Petit 2003). Conservation genetics focuses on the diversity, differentiation and structure of genetics, kinship, effective population size, and Evolutionary Significant Unit (ESU) of a species based on biodiversity. The study of these elements can provide information on biodiversity at multiple hierarchical levels. With the warming of global climate, its impact on endangered plants is also increasing. The primary goal of conservation genetics is to protect the genetic diversity of species and their evolutionary potential. Genetic variation is the basis of species evolution, and a species can adapt to complex and variable environments only by accumulating enough genetic variation (Frankel and Frankel 1981). Studies of endangered species can improve the understanding of genetic distribution within and among populations to establish efective strategies for species conservation and management (Herick and Miller 1992). In addition, population dynamic history analysis can establish an evolutionary clade model and then verify its probability. Full understanding of the genetic diversity, genetic variation, genetic structure, and the current state of population dynamics is essential to reveal the evolutionary history and adaptation potential of species (Yang et al. 2014). The evolutionary potential of a species in nature depends on its level of genetic variation and diferentiation. Furthermore, knowledge of genetic variation and evolutionary history is critical for effective conservation and management of an endangered species.

Tetracentron sinense Oliver (*T. sinense*), the only living species in the Tetracentraceae family, is mainly located in deciduous forests in central and southwest China (Fu 1992). As an Arcto–Tertiary relict plant with rich fossil records from the Eocene of Cenozoic (53–36.5Ma) period (Wu 2004), *T. sinense* is important for the study of ancient flora and the origin and evolution of angiosperms. It flowering occured in the rainy season from June to July and pollinated by insects and winds, which limits its dispersion distance. Comparison of the fossils distribution with the retreat living population showed that their historical distribution is certified each other. Sun et al study showed that *T. sinense* historical distribution in the northern hemisphere, and even the Arctic, and now only distributed in central and southern China (Sun et al. 2014). What is the reason for such a huge difference in distribution? In recent years, climate change during the Quaternary ice age and anthropogenic activities such as overgrazing and deforestation threatened living populations of *T. sinense* and led to a sharp decline in effective population size (Slatkin 1987). Thus, *T. sinense* is currently listed in Appendix III of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora (https://cites.org/eng/node/41216)) and is also listed as a National Second–grade Key Preserved Wild Plant in China (Fu and Jin 1992). Our study comply with IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. To restore and recover the degraded habitats and design and implement appropriate conservation strategies of *T. sinense*, exploration of genetic diversity within and among populations investigation of the population differentiation and population dynamics is very important.

Pollination ecology, seed and seedling ecology, community ecology and the physiological ecology of photosynthesis have been studied to explore the causes of environment–related endangerment and suggest conservation strategies for the germplasm resources of *T. sinense* (Cao et al. 2012; Gan et al. 2013; Han et al. 2015; Li et al. 2018; Tian et al. 2018; Fan et al. 2021; Lu et al. 2021). The conservation genetics of natural *T. sinense* populations were refering the chloroplast DNA and inter–simple sequence repeat (ISSR) markers (Sun et al. 2014). Due to a slow evolutionary rate and insufficient polymorphic sites (Muir and Filatov 2007), the maternally inherited chloroplast genes must be combined with nuclear DNA markers to reveal the genetic variation within populations of *T. sinense*. In addition, due to their dominance, ISSR markers do not distinguish between homozygous and heterozygous individuals nor fully reveal the status of genetic variation (Ye et al. 2009). Thus, developing new genetic markers to reveal the actual genetic variations in *T. sinense* is urgent and necessary. Nuclear simple sequence repeat (SSR) markers have been widely used as molecular markers in many research fields, such as fingerprint identification, paternity analysis, population genetic structure analysis, genetic map construction, comparative genome analysis, and molecular marker–assisted breeding (Powell et al. 1996; Galperin and Koonin 2001; Schlotterer 2001; Wyman et al. 2003; Biradar et al. 2004; Labbé et al. 2008). Therein, SSR markers have been widely applied to important plant studies to understand the extent and patterns of genetic variation, the dynamic history and the unidentified genetic potential of plant species amid a changing environment. In recent years, the development of genome sequencing technology led to a study on chromosome–level genome assembly for the Tertiary relict plant, *T. sinense*. The results found that *T. sinense* has a small genome size (986.3 Mb) with high frequency repetitive elements, which is the most

important factor affecting genome size (Li et al. 2021). Study results also showed that the effective population size of *T. sinense* is continuously small due to the influence of the Quaternary ice ages. 50 *T. sinense* individuals were divided into three evolutionary branches, which is similar to the results generated through genome resequencing (Li et al. 2021).

In the following study, 22 natural populations of *T. sinense* were used to determine genetic diversity, genetic differentiation, genetic structure and population dynamic history based on the 14 developed SSR molecular markers. The aims of the study were: (i) to identify genetic diversity and genetic differentiation within and among populations and to identify whether any factors such as inbreeding, genetic drift or genetic bottlenecks may have reduced levels of genetic diversity, (ii) to define the natural populations evolutionary model and to clarify the differentiation model and differentiation time of the *T. sinense* populations, and (iii) to advise the design and implementation of appropriate conservation strategies to manage these significant germplasms.

Material and Methods

Plant samples

The natural populations of *T. sinense* are mainly distributed in central and southwest China. According to the Chinese Virtual Herbarium (CVH), 193 individual samples were obtained from 22 natural populations across the *T. sinense* distribution range in China from May to July 2018 (Table 1, Fig. 1). Due to excessive destruction and poor regeneration, few existing native population resources were available for study. Thus, approximately ten samples were collected per population. Fresh, tender leaves were collected, immediately dried with silica gel in zip lock plastic bags, transported back to the laboratory, and stored in a freezer at -80°C. The sampled individuals were kept at least 100 m to avoid sampling clonal individuals or individuals from the same family. Prof Xiao–Hong Gan undertook the formal identification of the plant material in this study. Voucher specimens were deposited in the herbarium of China West Normal University (ID: MCS202006). Detailed sampling information is provided in Table 1.

Table 1
Collection information of 22 *T. sinense* populations.

Population	Code	Location	Number	Longitude (E)	Latitude (N)	Altitude (M)
LX–HK	D1	Longxi–hongkou, Sichuan	11	103.581	31.147	1750
EMM	D2	Emei mountain, Sichuan	5	103.356	29.566	1799
DFD	D3	Dafengding, Sichuan	10	103.143	28.770	2280
MCM	D4	Micang mountain, Sichuan	10	106.571	32.659	1894
TJH	D5	Tangjiahe, Sichuan	9	104.649	32.609	1996
FTZ	D6	Fengtongzhai, Sichuan	8	102.866	30.671	2433
JFM	D7	Jinfo mountain Chongqing	7	107.183	29.037	2099
FJM	D8	Fanjing mountain, Guizhou	9	108.692	27.908	2101
KKS	D9	Kuankuoshui, Guizhou	10	107.166	28.233	1618
LGM	D10	Leigong mountain, Guizhou	10	108.209	26.378	1779
DSH	D11	Dashahe, Guizhou	4	107.769	29.152	1629
SHM	D12	Shunhuang mountain, Hunan	10	110.011	26.371	1692
BDGM	D13	Badagong Mountain, Hunan	9	110.068	29.772	1389
QZMM	D14	Qizimei mountain, Hubei	7	109.738	30.035	1525
SNJ	D15	Shennongjia, Hubei	10	110.365	31.432	1666
WFHH	D16	Wufenghouhe, Hubei	10	110.545	30.072	1406
FP	D17	Foping, Shanxi	10	107.808	33.640	1639
HBY	D18	Huangbaiyuan, Shanxi	7	107.559	33.808	2043
BSJ	D19	Baimahe, Gansu	10	104.336	32.896	2224
KP	D20	Kangpu, Yunnan	8	99.137	27.558	2568
BMXM	D21	Baimaxue mountain, Yunnan	10	99.361	27.652	2678
GLGM	D22	Gaoligong mountain, Yunnan	9	98.711	25.979	2486

SSR primer information, DNA extraction and amplification

A total of 121,022 SSR loci obtained in 114,350 SSR–containing sequences were identified based on RAD–seq. Primer3.0 was used to design the sequence fragments containing SSR (Rozen and Skaletsky 2000; Andreas et al. 2012). Fourteen SSR primers were screened according to nucleotide polymorphisms

(*PIC*) and synthesized by Shanghai Meiji Biotechnology (China) for this study (Table S1). The RAD–sequencing was completed in another study.

Total genomic DNA was extracted from approximately 50 mg of frozen leaves using LABGENE™ Plant DNA Isolation Kit for Dry Sample (LABGENE Biotechnology Co., Ltd., Chengdu, China). DNA concentration was determined by comparing the sample with a reference DNA on 0.8% agarose gel and NanoDrop2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). DNA was stored at -20℃ for SSR amplification.

The optimal reaction system was as follows: 12.5 μl 2 × Taq PCR Master Mix, 1 μM primer, 70 ng genomic DNA, and double distilled water (to 12.5 μl) in a total volume of 25 μl. PCR conditions were as follows: 94°C for 3 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing for 30 s, DNA elongation at 72°C for 1 min, and final extension at 72°C for 5 min; the reaction products were kept at 12°C. Amplified products were separated by electrophoresis on 8% polyacrylamide gels. The 50 to 500 bp DNA ladder (LABGENE Biotechnology) was used as molecular weight standard.

Data analysis

1. Parameters of genetic diversity

Genetic diversity within and among populations were measured through the observed alleles (*Na*), effective number of alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Nei's Diversity Index (*h*), Shannon's Information Index, percentage of polymorphic loci (*PPL*), and gene flow (*Nm*). Ewens–Watterson neutral detection measured whether the SSRs followed neutral evolutionary patterns or not; this was measured by the POPGENE program (Yeh et al. 1999).

2. Population differentiation and structure analysis

We used the program Arlequin version 3.11 to conduct an analysis of molecular variance (AMOVA) and to estimate the genetic variation that was assigned within and among populations (Excoffier et al. 2006). STRUCTURE Version 2.3.4 was run with different K values ranging from 1 to 10 based on a Bayesian analysis to characterize the genetic structures of the 22 populations (Pritchard et al. 2000). Ten independent runs per K were performed with a 1,000,000–length burn–in period and 1,000,000 Markov chain Monte Carlo (MCMC) replicates after the burn–in. The STRUCTURE calculation results (Results. Zip) were submitted to the online tool, Structure Harvester, to determine the number of clusters in 22 populations (Earl and Vonholdt 2012).

3. Bottleneck analysis

For microsatellites, Spencer et al. (2000) suggested combining allelic diversity with expected heterozygosity for assessment of genetic bottlenecks in populations (Spencer et al. 2000). Allelic diversity is more sensitive than heterozygosity, reflecting an immediate consequence emphasized by genetic bottlenecks (Nei 1973; Spencer et al. 2000). The computation was performed under a stepwise mutation model (SMM) and a two-phased model (TPM) of the program BOTTLENECK Version 1.2.02

with the default parameters to detect whether *T. sinense* populations had undergone genetic bottlenecks. We did not use the standardized differences test in this study because the test was usually used at the condition of having at least twenty polymorphic loci (Kimura and Ohta 1978; Luikart et al. 1998; Cristescu et al. 2010; Ndo et al. 2010; Haanes et al. 2011).

4. Construction of the population dynamic history models

The approximate Bayesian computation (ABC) method, implemented in DIYABC v 2.1.0 (Jean–Marie et al. 2014), was used to analyze the population demographic history. Based on the fndings from STRUCTURE analysis, three populations were defned (Fig. 2). The Pop 1 was composed of nine targeted sites (LX–HK, DFD, MCM, TJH, FTZ, JFM, LGM, DSH and SHM). The Pop 2 was composed of nine targeted sites (EMM, FJM, KKS, BDGM, QZMM, SNJ, WFHH, BMXM and GLGM). The Pop 3 was composed of nine targeted sites (FP, HBY, BSJ and KP). We assumed no migration among populations in each scenario. In scenario 1, the three populations of Pop2 and Pop3 were merged at time ta and Pop3 at time td. In scenario 3, the three populations of Pop3 and Pop1 were merged at time ta and Pop2 at time td. In scenario 4, the three populations of Pop1, Pop2, and Pop3 merged simultaneously at the same time ta. Finally, in scenario 5, the Pop3was assumed to originate from an admixture of the Pop1 and Pop2 at time ta. The rate of admixture from the Pop1 and Pop2 was set as “ra” and the one from Pop3 to Pop1 was set as “1-ra”. The Pop2 merged Pop1 at time td. The analysis of *Rhododendron meddianum* dynamic history modeling informed our study’s analysis (Jean–Marie et al. 2014). The posterior probabilities were determined following 1,000,000 simulations for each scenario. The goodness–of–fit of the three scenarios were also assessed by a principal component analysis (PCA) using the option “model checking” in DIYABC software.

Results

Genetic diversity of 22 T. sinense populations

102 alleles were detected by the 14 SSR loci across the 193 individuals from 22 populations, Original gel of Primer 61 is presented in Supplementary Fig S1. At the species level, the value of *Na*, *Ne*, *I*, *Ho*, and *He* were 7.429, 4.435, 1.631, 0.442, and 0.559, respectively (Table S1). At the population level, the value of *Na*, *Ne*, *I*, *Ho*, and *He* were 3.221, 2.505, 0.937, 0.434, and 0.566, respectively. The genetic diversity of DFD, TJH, FTZ, FJM, LGM, BDGM, SNJ, WFHH, BSJ and BMXM populations are positive than 1 with high level. The Fixation Index (*Fis*) value was − 0.082 (Table 2). Ewens–Watterson neutral test results showed that most polymorphic sites did not deviate significantly, which is essentially consistent with the neutral evolution model (Table S2).

Table 2
Genetic diversity of 22 *T. sinense* populations.

Population	*Na*	*Ne*	*I*	*Ho*	*He*	*Fis*
LX–HK	3.214	2.313	0.939	0.481	0.519	0.077
EMM	2.714	2.562	0.911	0.381	0.619	− 0.131
DFD	3.643	2.606	1.008	0.416	0.584	− 0.102
MCM	2.857	2.352	0.863	0.513	0.487	0.005
TJH	3.714	2.649	1.041	0.528	0.473	0.157
FTZ	3.500	2.851	1.059	0.415	0.585	0.006
JFM	2.714	2.397	0.816	0.368	0.632	− 0.367
FJM	3.500	2.740	1.033	0.453	0.547	0.021
KKS	3.071	2.284	0.836	0.483	0.517	− 0.084
LGM	3.929	2.757	1.080	0.519	0.482	0.133
DSH	2.571	2.19	0.796	0.304	0.696	− 0.397
SHM	2.857	2.254	0.791	0.456	0.544	− 0.229
BDGM	3.429	2.910	1.038	0.332	0.668	− 0.187
QZMM	3.071	2.600	0.96	0.253	0.747	− 0.362
SNJ	3.857	2.580	1.063	0.442	0.558	− 0.012
WFHH	3.643	2.744	1.079	0.250	0.750	− 0.261
FP	2.846	2.146	0.831	0.525	0.475	0.095
HBY	2.231	1.952	0.657	0.444	0.556	− 0.214
BSJ	3.929	2.939	1.149	0.542	0.458	0.242
KP	2.857	2.149	0.797	0.495	0.505	− 0.088
BMXM	4.000	2.944	1.121	0.417	0.583	0.017
GLGM	2.714	2.189	0.737	0.540	0.460	− 0.125
Mean	3.221	2.505	0.937	0.434	0.566	− 0.082

Note: *Na*, Number of alleles; *Ne*, Number of effective alleles; *I*, Shannon's information index; *Ho*,Observation of Heterozygosity; *He*, Expected Heterozygosity; *h*, Nei's Diversity Index; *Fis*, wright's fixation Index.

Genetic variation and genetic differentiation of 22 T. sinense populations

AMOVA analysis showed that the genetic variation within populations accounted for 84% of the total variance, while the genetic variation among populations accounted for only 16% of the total variance. This indicates that the most genetic variation of *T. sinense* came from within populations (Table 3). The genetic differentiation coefficient (*Fst*) at the species level was 0.31, indicating a significant genetic differentiation among populations (Weir and Cookerham 1984). Furthermore, the Nm at the species level was 0.555, indicating that genetic drift was the main cause of genetic differentiation (Table S3).

Table 3
The AMOVA analysis of *T. sinense.*

Source of variation	DF	SS	Variant components (%)
Among Populations	21	236.483	16
Within Populations	171	738.672	84
All	192	975.155	100
	Fst = 0.16		
Notes: *DF*, degree of freedom; *SS*, Sum of Squares of Deviations.			

Genetic structure of 22 T. sinense populations

STRUCTURE analysis showed that there was an obvious inflection point and the maximum value was obtained when K = 3. This indicates that the *T. sinense* populations could be divided into three clusters (Fig. 3a, Fig. 3b). Cluster 1 included the 9 populations of LX–HK, DFD, MCM, TJH, FTZ, JFM, LGM, DSH and SHM, cluster 2 was composed of the 9 populations of EMM, FJM, KKS, BDGM, QZMM, SNJ, WFHH, BMXM and GLGM, and cluster 3 was composed of the 4 populations of FP, HBY, BSJ and KP (Fig. 3c).

Genetic bottleneck of 22 T. sinense populations

When SMM was used, only SNJ (*Na* = 3.86, *Ne* = 2.58, *Ho* = 0.44, *He* = 0.56) showed a significant excess of heterozygosity. When TPM was used, only FTZ (*Na* = 3.50, *Ne* = 2.85, *Ho* = 0.42, *He* = 0.59) had a significant excess of heterozygosity as estimated with the two methods ($P < 0.05$), suggesting that SNJ and FTZ deviated from mutation-drift equilibrium and indicate that there was a past reduction of effective population size in the species (Table 2, Table 4). Populations of *T. sinense* underwent a demographic bottleneck in history.

Table 4
Models of Bottleneck Detection of *T. sinense*.

Population	SMM		TPM	
	Sign test	Wilcoxon test	Sign test	Wilcoxon test
BSJ	0.131	0.735	0.534	0.497
BMXM	0.410	0.761	0.388	0.194
DFD	0.166	1.000	0.220	0.217
FJM	0.558	0.946	0.545	0.455
FP	0.598	0.791	0.562	0.380
FTZ	0.094	0.058	0.028*	0.009*
KKS	0.594	0.946	0.535	0.735
KP	0.603	1.000	0.425	0.588
LGM	0.523	0.903	0.422	0.268
LX–HK	0.456	0.502	0.207	0.194
SHM	0.421	1.000	0.424	0.465
SNJ	0.007*	0.042*	0.196	0.268
TJH	0.465	1.000	0.562	0.244
WFHH	0.315	0.839	0.572	0.414

Notes: *SMM*, Step mutation model; *TPM*, Two phase mutation model; *: $P < 0.05$.

Notes: a, the trends of Ln P (D)(± SD) against K; b, the trends of ΔK against K; c, The clustering results of STRUCTURE(K = 3).

Demographic history of 22 T. sinense populations

Based on the generated genetic structure, three types of evolutionary models were established, including branching differentiation model, radial differentiation model and common differentiation model (Fig. 2, Fig. 4). Using DIYABC software to speculate on the differentiation time of different clusters, scenario 4 was determined to be the most suitable for the evolutionary model of the nuclear gene of *T. sinense* as the three clusters have common ancestral population. Each group presents a pattern during evolution. The principal component analysis (PCA) of the simulated dynamic model showed that scenario 4 accounts for 30.9% (Fig. 4a, 4b, 4c), indicating the results are reliable. A posteriori probability results of direct estimation were the same as those calculated through logistic regression (Fig. 5a, 5b), which supports scenario 4 as the best evolutionary model. Supposing the generation time of *T. sinense* was 50 to 100 years for a generation, the time of differentiation is 1.115×10^4 to 2.23×10^4 years ago (ta = 223),

the size of the ancestral group is 2.73×10^4 (NA) and the total size of the progeny population is 1.043×10^4 (N1 + N2 + N3). The results showed that glaciers and cold climate have become the main factors influencing the existing genetic structure of the *T. sinense* population with the deterioration of the climate of Quaternary glaciation.

Discussion

Genetic diversity within species and population level

In general, plant species with higher genetic diversity have greater ability to adapt to environmental changes. Therefore, understanding the level of genetic diversity within and among populations is essential to establish conservation strategies. Shannon's Information Index and expected heterozygosity are commonly used to estimate within–population genetic diversity. For the endangered speceis, the study based on 14 SSR loci, the genetic diversity of *T. sinense* within–population (*I*: 0.937, *He*: 0.566) was higher than *Taxus chinensis* var. *Mairei* (*I*: 0.862, *He*: 0.459), *Ottelia alismoides* (*I*: 0.08, *He*: 0.052), *Sinowilsonia henryi* (*I*: 0.873, *He*: 0.537) (Wen et al. 2012; Wang and Zhou 2019; Wagutu et al. 2021). These species are all endangered species, which is of great significance to determine the level of genetic diversity. Conversely, the within–population genetic diversity was lower than *Firmiana danxiaensis* (*I*: 0.949, *He*: 0.536), *Glycyrrhiza uralensis* (*I*: 1.354, *He*: 0.692) and *Elaeagnus mollis* (*I*: 0.981, *He*: 0.515) (Li et al. 2017; Xin 2019; Ye 2019), indicating a relatively moderate level of genetic diversity of *T. sinense*. As far as other endangered plants in the same distribution (Central and southern China) are concerned, no matter at the species level or the population level, showed the high genetic diversity and genetic variation as the endangered species such as *Sassafras tzumu* (Hemsl.) Hemsl. (*He*: 0.317), *Ficus hirta* Vahl. (*He*: 0.706), *Rhododendron longipedicellatum* (*He*: 0.507) and *Michelia shiluensis* (*He*: 0.718) (Deng et al. 2020; Cao et al. 2022; Lu et al. 2022; Wang et al. 2022). The results revealed high genetic diversity at the species level (*I*: 1.631, *He*: 0.559) and within populations (*I*: 0.937, *He*: 0.566).

The genetic diversity of plant species is related to distribution range, population size, life cycle, mating system, gene flow and fossil record (Grímsson et al. 2008; Forrest et al. 2017). Of these factors,the distribution range and a small population size tend to decrease genetic diversity (Ellstrand and Elam 1993). The historical distribution of *T. sinense* covered an area close to the Arctic, whereas the current distribution is present only in central and south China. This distribution range likely led to lowered genetic diversity in *T. sinense* populations. The average *Fis* of overall populations for each locus was − 0.061. Five of the studied fourteen loci had a positive fxation index, indicating an excess of homozygotes and inbreeding. Of these, however, only one loci had signifcant inbreeding ($p < 0.01$). The Three loci had negative values and no signs (Angeloni et al. 2011).

Genetic variation, genetic differentiation and population genetic structure

Natural selection is the most important evolutionary force leading to population differentiation, and gene flow is an important factor opposing selection. The results of AMOVA analysis showed that the genetic variation of *T. sinense* occurred mainly within the population (84%), which was much higher than *Aristolochia delavayi* (68.4%), *Paeonia Decomposita* (73.48%), *Rhododendron meddianum* (80.12%) (Wang 2020; Yu et al. 2021; Zhang et al. 2021), lower than *Michelia shiluensis* (89.92%), *Ferula tadshikorum* (93.8%) (Xin 2019; Deng et al. 2020). These findings demonstrate that *T. sinense* individuals within the population retained relatively high genetic potential. This might be attributed to geographical barriers. Gene flow strongly influences the genetic structure and genetic differentiation of populations (Hamrick 1982). In this study, the degree of gene flow (*Nm*) was indirectly estimated based on Fst values across all populations for each locus. The degree of Nm among *T. sinense* populations was 0.555 indicating that there is a large differentiation among populations (*Nm* < 1), which was also lower than *Corylus mandshurica* (*Nm* = 1.808), *Taxus wallichiana* var. *mairei* (Taxaceae) (*Nm* = 1.76) and *Prunus sibirica* (*Nm* = 3.595) (Zhang and Zhou 2013; Wang et al. 2014; Zong et al. 2015). The gene flow in this study was similar to *Origanum compactum* (*Nm* = 0.88), *Tapiscia sinensis* (*Nm* = 0.7274) and *Aristolochia delavayi* (*Nm* = 0.591) (Freeland 2005; Zhou et al. 2016; Aboukhalid et al. 2017). The results showed a higher level of genetic drift and inbreeding for *T. sinense*, which generally leads to a significant loss of genetic diversity within populations, an increase in genetic differentiation among populations (Ellstrand and Elam 1993; Willi and Hoffmann 2006), and a reduction in gene flow consisting with this study. These population–level findings may be related to the limited reproductive characteristics of *T. sinense*. The flowering period of *T. sinense* coincides with rainy seasons, which leads to decreased pollination efficiency due to strong winds and insects (Lu et al. 2021). Additionally, the seeds of *T. sinense* usually disperse around their mother trees (Li et al. 2015). Thus, distance–limited pollen flow and short–distance seed dispersal significantly influence gene flow in *T. sinense*.

Structure analyses showed that the 22 populations of *T. sinense* were assembled into three groups, clustered in the northeast, middle and southwest geographical areas. This was also reflected in the results from the ISSR markers (Li et al. 2018). The three groups of *T. sinense* were bounded by the Hengduan and Qinling Mountains, where some higher positions can be described as refuges (Shen et al. 2002; Chen et al. 2011). The environmental condition of the Qinling Mountains is complex, and the temperature, climate and topography highly differ between the north and the south (Shi et al. 2018). In particular, the watershed between the north and the south of China may influence the distribution of *T. sinense*. In addition, a number of genetically similar populations, such as *Cercidiphyllum japonicum* and *Taxus chinensis* var. *Mairei*, were located in the same geographic region (Xie 2013; Zeng 2021).

Genetic bottleneck and demographic history

The Fis value of the 22 populations was − 0.082, indicating excess heterozygosity, homozygous deletion, and a high degree of variation. Therefore, we inferred that *T. sinense* populations experienced special events in some historical period, such as mass extinction during Ice Age. Bottleneck analysis showed that the FTZ and SNJ populations have recently experienced a bottleneck effect. Dramatic changes in Pleistocene climate including several glacial and interglacial transitions have had profound influence on

the demographic and evolutionary history of species (Hewitt 2000; Davis and Shaw 2001). Based on the experience of long–term experiment process, the generation time of *T. sinense* are estimated from 50 to 100 years. Therefore, the time of differentiation was 1.115×10^4 to 2.23×10^4 years (ta = 223), occurring during the last ice age (Clark et al. 2009). The size of the ancestral group is 2.73×10^4 (NA), and the total size of the progeny population is 1.043×10^4 (N1 + N2 + N3). These results reflected the considerable population decline (NA > N1 + N2 + N3) of *T. sinense*. This differentiation pattern can be explained by two hypotheses. The first posits that isolation leads to differentiation. Due to a variety of geological and climatic changes, the flora was disrupted, resulting in an uneven distribution of the ancient flora in Europe, East Asia, Western North America and Eastern North America. Previous studies had shown that climate cooling led to the divergence of *T. sinense* (Sun et al. 2014). With the Quaternary gas ice age and volatile climate, the original broad and continuous distribution area of *T. sinense* has been gradually broken into three small, independent areas (Zachos et al. 2008; Nagalingum et al. 2011). This also may explain the pattern of simultaneous differentiation of the three clusters of *T. sinense* with a common ancestral species. The premise of this explanation is that the ancestral quantities of *T. sinense* are much larger than the progeny population. Previous studies have found that the fossil materials are much more than the existing materials recorded, which indirectly verifies the reliability of this hypothesis (NA > N1 + N2 + N3). The second is the migration hypothesis, which states that the population size of *T. sinense* changed sharply during the Quaternary age climate deterioration. The fossil materials of Tetracentron were discovered in the Cenozoic Eocene in the western part of China, the northernmost part in the southern part of Gansu and the eastern part in Henan. According to the migration hypothesis, *T. sinense* moved to the southwest, central and northeast regions almost at the same time, forming the differentiation pattern obtained by the ABC simulation. Although this hypothesis is consistent with the data associated with the fossil material findings of *T. sinense*, the cold climate, and the barrier of high mountains and river flow make the hypothesis less likely to be supported.

Conservation implications

In wild populations of *T. sinense*, human disturbance and habitat fragmentation are an important cause of low level genetic diversity (Gan et al. 2013). Therefore, priority measures should be taken to protect the existing populations and *T. sinense* habitats through conduction of in–situ conservation. Some suggest that populations like BSJ and WFHH should be prioritized for in–situ conservation because of their relatively higher genetic diversity. Alternatively, ex–situ conservation strategies for restoring genetic diversity of LGM populations, such as germplasm resource collection and artificial gene flow for population rejuvenation may be considered to avoid the loss of genetic resources.

Our study on the genetic structure of *T. sinense* provides important conservation implications for this narrowly distributed species. Reflected by the study of historical population dynamics, each group is a differentiated branch. Therefore, each group should be considered a conservation and management unit. Increasing hybridization of individuals among populations within the same group may contribute to increased population size and appropriate improvement in genetic potential.

Conclusions

SSR molecular marker analysis showed that *T. sinense* is characterized with a high level of genetic diversity, indicating maintenance of evolutionary and adaptive potential. Genetic variation of *T. sinense* occurred mainly within the population. A significant genetic differentiation was detectable among natural populations of *T. sinense*. STRUCTURE software divided 22 populations into 3 clusters, categorizing the Hengduan and Qinling Mountains as two major glacial refuges. The DIYABC simulation showed that the three groups in southwest, central and northeast China differentiated at the same time, and the differentiation occurred between 1.115×10^4 and 2.23×10^4 years ago during the last ice age. We speculate that the three clusters of *T. sinense* population began to evolve in these three regions at the same time as the climate deterioration in the Last Glacial Maximum, forming this differentiation model. The results of this study are important for the development of scientific conservation strategies, such as in–situ conservation for existing populations and their habitats. Populations with high genetic diversity and genetic variation (BSJ and WFHH) conduct in–situ conservation, whereas populations with low genetic diversity (LGM) conduct ex–situ conservation. The population demographic history obtained in this study will provide the scientific basis for these conservation efforts.

Declarations

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Competing Interests

The authors declare that they have no conflict of interest.

Author Contributions

All authors contributed to the study conception and design. Zhongqiong Tian: Formal analysis; Writing–original draft; Fan Duan: Investigation; Validation; Weili Mao: Investigation; Validation; Qiong La: Investigation; Validation; Xiaohong Gan: Funding acquisition; Investigation; Project administration; Supervision; Visualization; Writing–review & editing.

Data Availability

The datasets in our study is deposited on Figshare (https://figshare.com/) with the DOI of 10.6084/m9.figshare.22218577

Compliance with Ethical Standards

The Nature Reserve Authority granted permissions to Professor Xiao-Hong Gan for using the endangered species of plant (*Tetracentron sinense*).

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Figures

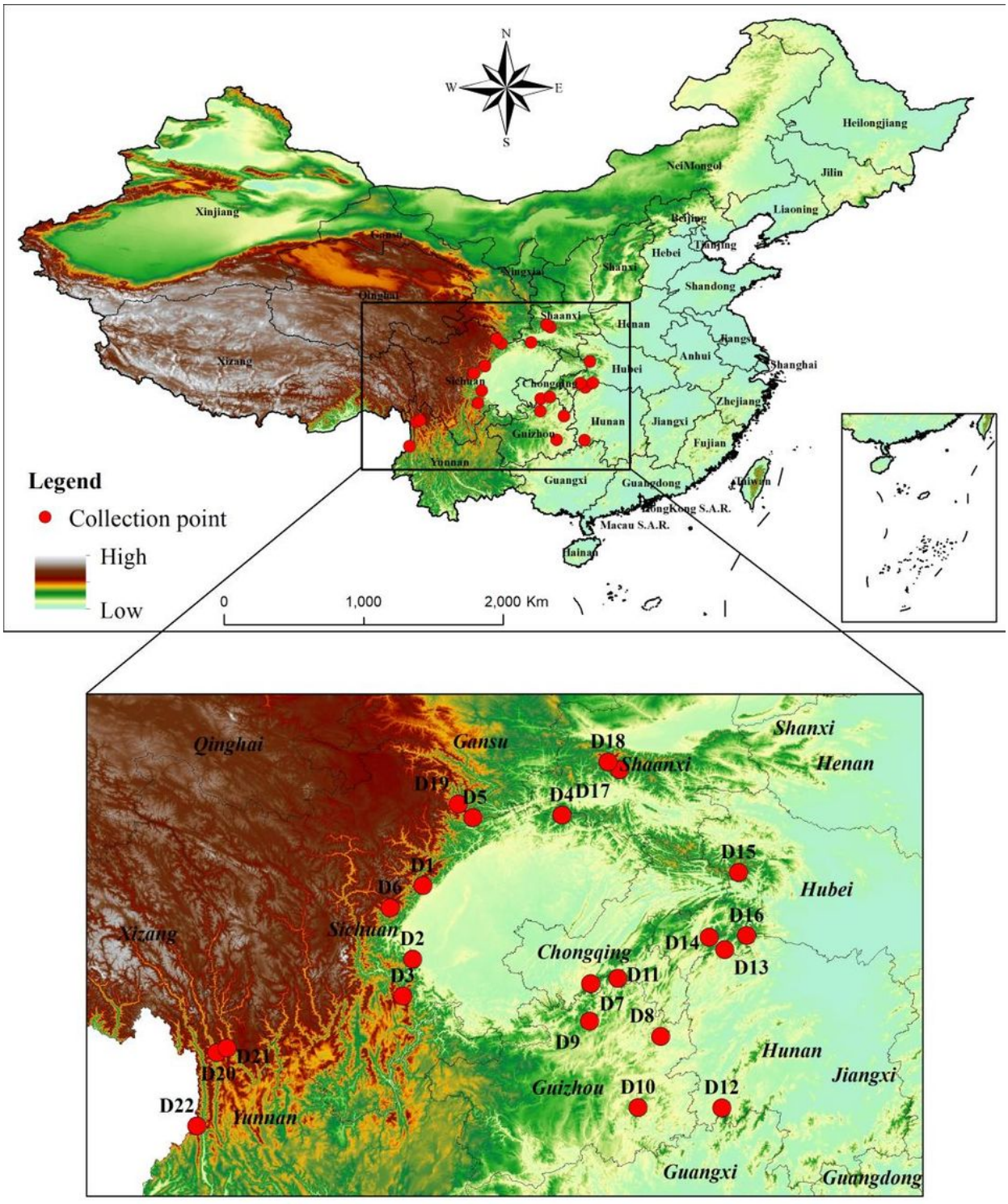

Figure 1

Field distribution of 22 *T. sinense* populations.

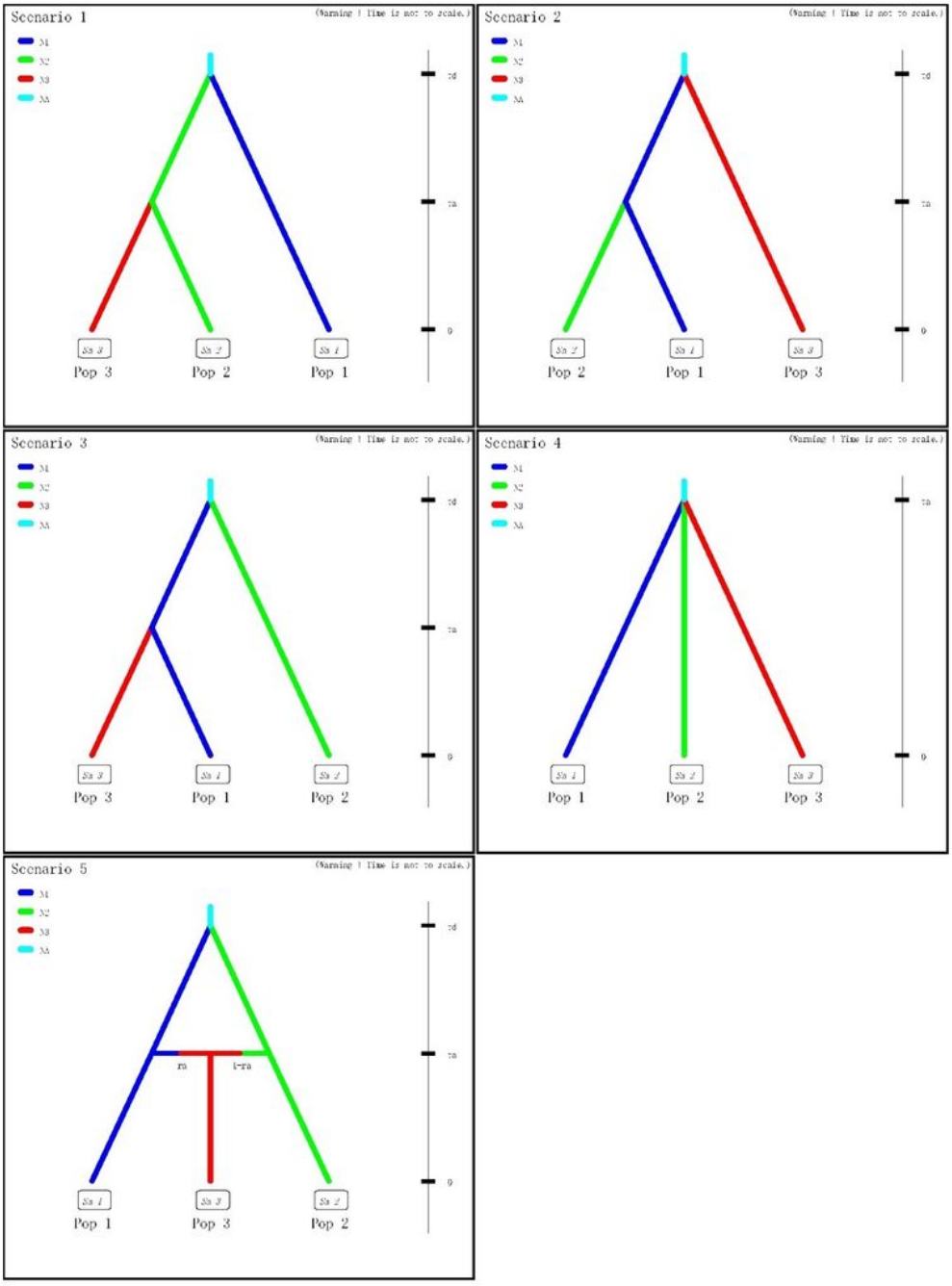

Figure 2

Five scenarios for *T. sinense* based on Approximate Bayesian Computation (K = 3) N1, N2, N3, NA represent effective population size, respectively. ta, td represent generation

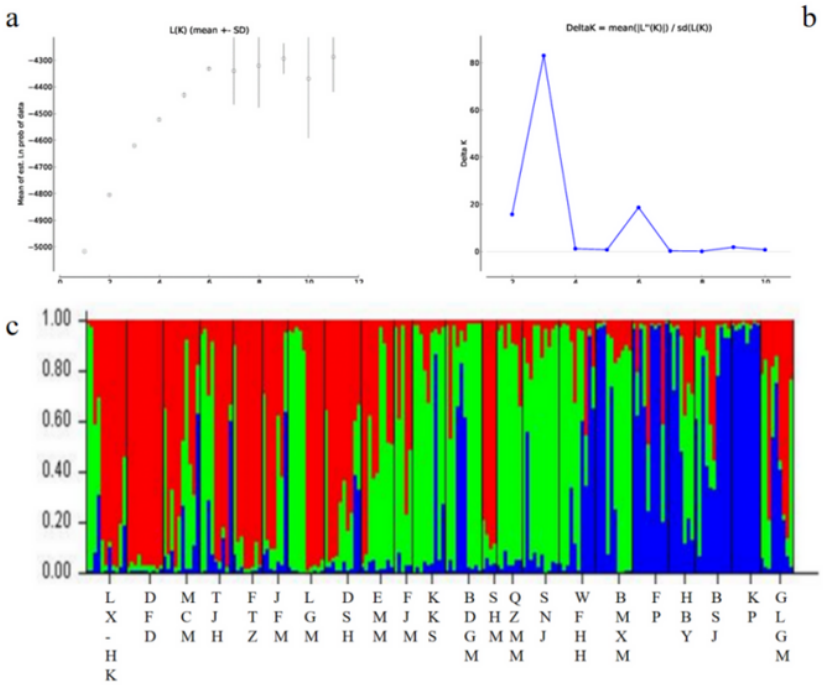

Figure 3

The results of STRUCTURE(K=3) analysis.

Notes: a, the trends of Ln P (D)(±SD) against K; b, the trends of ΔK against K; c, The clustering results of STRUCTURE(K=3).

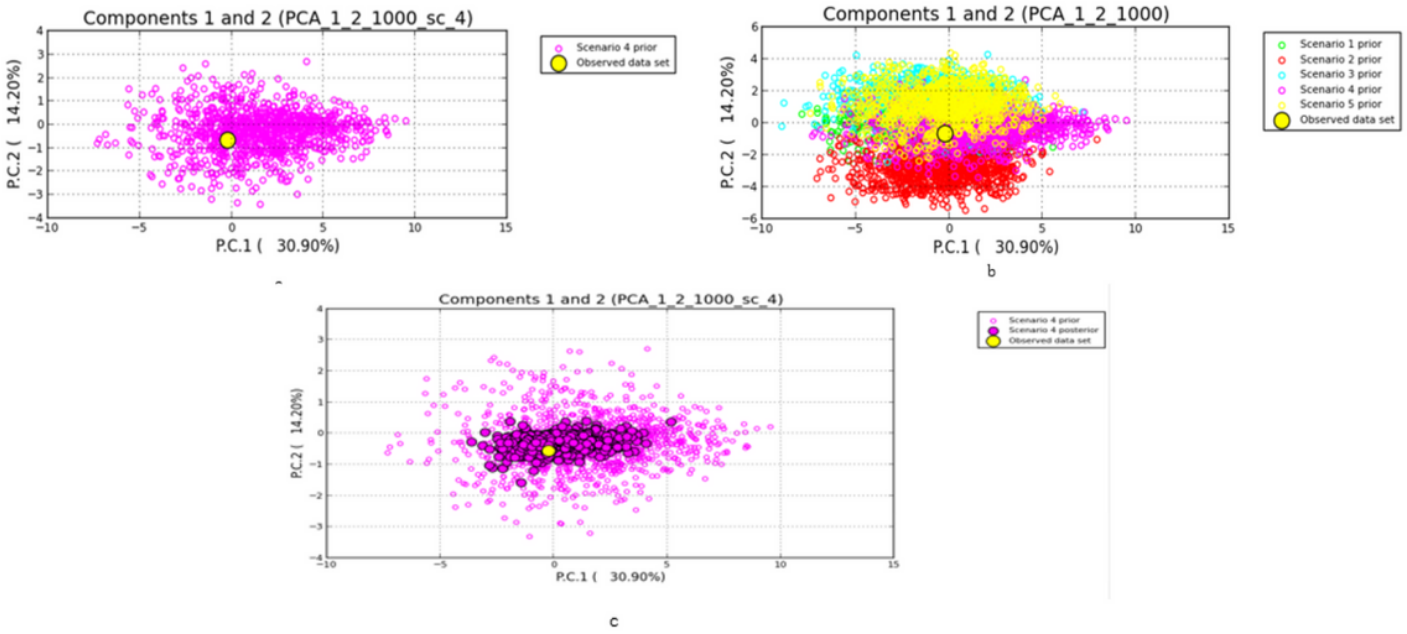

Figure 4

Five Dynamic Modelling Principal Component (PCA) Statistics analysis.

Note: a, distribution statistics for the fourth scennario; b, total simulation of all 5 scennarios; c, comparison of the actual results of the fourth scennario. The yellow dots in the figure represent the real data, and the small dots in other colors represent the data set in which the parameters in the posterior distribution are simulated.

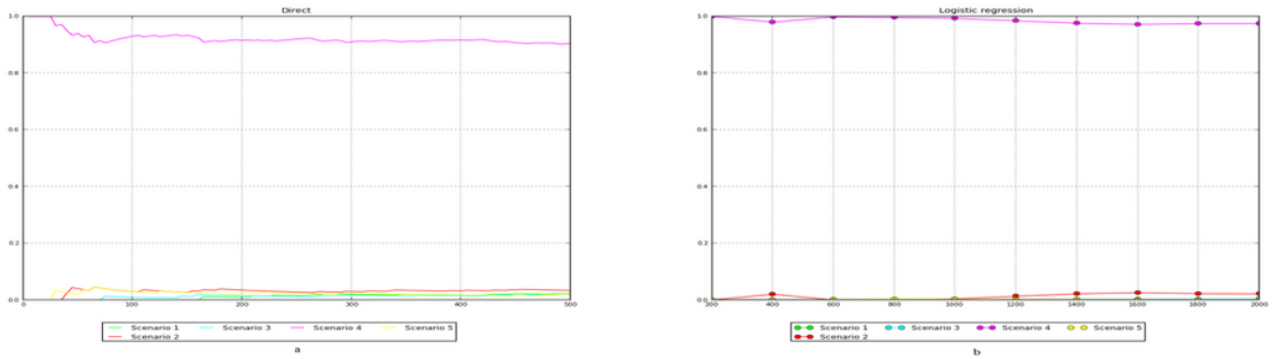

Figure 5

Posterior probabilities of 5 hypothetical historical dynamic models of *T. sinense.*

Note: a, results directly estimated; b, Results of logical regression estimation.

Supplementary Files

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- Supplymentaryfiles.zip