Pronounced genetic differentiation in *Fokienia hodginsii* revealed by simple sequence repeat markers

Qianyi Yin\(^1\) | Sufang Chen\(^1\) | Wei Guo\(^2\) | Yanshuang Huang\(^1\) | Yelin Huang\(^1\) | Renchao Zhou\(^1\) | Qiang Fan\(^1\) | Wenbo Liao\(^1\)

\(^1\)State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, Sun Yat-sen University, Guangzhou, China

\(^2\)Department of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, China

**Correspondence**

Wenbo Liao and Qiang Fan, School of Life Sciences, Sun Yat-sen University, Guangzhou, China.

Email: lsslwb@mail.sysu.edu.cn (WL); fanqiang@mail.sysu.edu.cn (QF)

**Funding information**

the Fourth National Survey on Chinese Material Medical Resources Program of State Administration of Traditional Chinese Medicine of the People’s Republic of China, Grant/Award Number: 2017-152-003; the Special Program for Key Basic Research of the Ministry of Science and Technology, China, Grant/Award Number: 2013FY111500; Chang Hungta Science Foundation of Sun Yat-sen University; National Natural Science Foundation of China, Grant/Award Number: 31570195 and 31670189; Natural Science Foundation of Guangdong Province, China, Grant/Award Number: 2016A030333326; Foundation of Jinggangshan Administration of Jiangxi Province, Grant/Award Number: 33000-7102993; Fundamental Research Funds for the Central Universities, Grant/Award Number: 16lgjc38

**Abstract**

*Fokienia hodginsii* is a Tertiary relict conifer of the monotypic genus *Fokienia* (Cupressaceae s.l.). Currently, the species is distributed in southern China, northern Vietnam, and northern Laos and listed as a “near threatened” species by the IUCN. In this study, a total of 427 individuals of *F. hodginsii* were sampled from China and Vietnam to characterize its genetic diversity and population differentiation. Based on the profiles of 12 simple sequence repeat (SSR) markers, we observed a high level of genetic diversity in *F. hodginsii* at the species level (\(H_e = 0.635\)), albeit slightly lower than that of its sister species *Chamaecyparis obtusa*. Signals of bottleneck events were detected in the populations GXDMS, GXHJ, V-PXB, and V-HB, probably due to Pleistocene glaciations or overexploitation in recent years. Pronounced genetic differentiation (\(F_{st} = 0.157\)) was found in this species. The inbreeding index (\(F_is = 0.176 \pm 0.024\)) indicated that *F. hodginsii* has a mixed mating system. Significant correlation was found between the pairwise genetic differentiation and geographic distance (\(r = 0.882, p = 0.01\)), suggesting that genetic differentiation among the populations follows the model of isolation by distance (IBD). STRUCTURE analysis and principal coordinate analysis revealed that these populations were divided into four groups: the western China group located mainly in the Yunnan–Guizhou Plateau, the central China group located mostly in the Luoxiao Mountains and Nanling Mountains, the eastern China group located in the Wuyi Mountains and the Vietnam group containing two populations in Vietnam. The different terrains and elevations of populations may be the most likely factors leading to the differentiation between the western China group and the central China group, while the geographic isolation caused by the lack of appropriate habitats may greatly contribute to the differentiation between the central China group and the eastern China group. Based on the results, some conservation suggestions for this species are provided, such as establishing seed orchards and multiple nature reserves.

**Keywords**

conservation, endangered species, *Fokienia hodginsii*, genetic differentiation, microsatellite, southern China
1 | INTRODUCTION

Under current rapid global climate change, many endemic species are facing a high risk of extinction due to limited natural ranges resulting from genetic stochasticity or demographic, environmental, or other factors (Caughley, 1994; Gitzendanner & Soltis, 2000; Lande, 1993). It is vital to understand the genetic characteristics of these species, such as genetic diversity and population structure, for their management and the development of effective conservation strategies (Eckert, Samis, & Lougheed, 2008; Lesica & Allendorf, 2010).

The gymnosperm family Cupressaceae Bartling comprises approximately 22 genera and 150 species. Most of these species are Tertiary relict species that arose in the Jurassic (possibly as early as the Triassic), thrived in the Jurassic, and decreased in members continuously up to the present. It is also the only family of gymnosperms that is present on all continents except Antarctica (Yang, Ran, & Wang, 2012). However, except for Juniperus, Sabina, and Cupressus, most species in this family are locally endemic, and ensuring their survival under future climate change will require public and scientific attention.

The genus Fokienia Henry et Thomas (Cupressaceae s.l.) contains only one extant species, Fokienia hodginsii (Dunn) Henry et Thomas (Farjon, 2005; Figure 1). Fossil records show that Fokienia was widely distributed in the Northern Hemisphere in ancient periods: fossils in forms with foliage and attached seed cones of Fokienia were reported from the Paleocene in Saskatchewan, central Canada (McIver & Basinger, 1990); the Oligocene in Jilin, northeastern China (Guo & Zhang, 2002); and the Miocene in Zhejiang, eastern China (He, Sun, & Liu, 2012). However, this genus is currently distributed in only southern China, northern Vietnam, and northern Laos (Zheng & Fu, 1978).

In China, it occurs at elevations between approximately 1,000 and 1,800 m as a minor constituent of the subtropical evergreen (mixed) forest (Zheng & Fu, 1978). This conifer is a good landscape tree species with a beautiful shape and straight trunk (Huang et al., 2013) and is commonly cut down for building materials because of its light texture and material stability (Huang, Huang, Guo, & Zheng, 2015). Currently, this conifer is listed as “near threatened (NT)” as part of the International Union for Conservation of Nature Red List (IUCN 2004) and the National Secondary Protected Plants by Order of the Forestry Bureau and Ministry of Agriculture of China (https://www.gov.cn/gongbao/content/2000/content_60072.htm), the vulnerable species by the Information System of Chinese Rare and Endangered Plants (https://rep.ipilant.cn/protlist), National Secondary Protected Plants in China and a K-class protected plant species in Vietnam (Vuong, 2009).

Most recent studies on F. hodginsii mainly focused on seed breeding, nursery technology, plantation cultivation, essential oil extraction and development and utilization of other resources (Huang et al., 2013; Zhao, 2005). Only one paper mentioned the progress in genetics of F. hodginsii, according to Tam, Trang, and Hoa (2011), who investigated the genetic diversity and population structure of F. hodginsii in Vietnam by applying ISSR markers and showed that F. hodginsii maintained a low level of genetic variability and a high level of genetic differentiation. They supposed that human disturbance may play a key role in the present status of F. hodginsii by leading to the degradation and fragmentation of its habitats.

Simple sequence repeat (SSR; microsatellite) markers, codominant markers with good reproducibility and high variability, are one of the best tools to understand species genetic diversity and population structure (Wang, Huang, & Long, 2013). Based on transcriptome sequencing, we synthesized 108 SSR primers that were successfully amplified in F. hodginsii (Ding et al., 2017). Applying these SSR markers, we aimed to investigate the levels of genetic diversity and population structure of this species, which could provide some reliable information for the protection of this endangered species.

2 | MATERIALS AND METHODS

2.1 | Sample collection and DNA extraction

A total of 427 individuals of F. hodginsii were sampled from 24 locations across twelve provinces of China and Vietnam (Table 1; Figure 2). A Garmin GPS unit (GPSMAP 62sc, Taiwan) was used to record the sample geographic locations with a margin of 10 m. For each population, fresh leaves were collected from 5 to 23 randomly selected fully grown individuals, which were at least 30 m apart from each other. Then, the leaf tissues were dried by silica gel and stored in zip-lock plastic bags for DNA extraction. Voucher specimens for each population were all deposited in the Herbarium of Sun Yat-sen University (SYS).

Total DNA was extracted from dried leaf tissue using the modified CTAB method (Doyle & Doyle, 1987). For each population, two individuals were randomly selected for PCR amplifications with all 108 primers designed by Ding et al. (2017). Fluorescence was added to the 3’ end of the 12 SSR markers (Table 2) with the highest polymorphism levels, and PCR amplifications were performed for all 427 individuals, in which the annealing temperature for each primer was set to 52°C. The PCR products were first inspected in 1% agarose gel and then electrophoresed on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, California, USA).

**FIGURE 1** Photograph of Fokienia hodginsii
2.2 Data analyses

Linkage disequilibrium (LD) between pairs of loci and deviation from Hardy–Weinberg equilibrium (HWE) for each locus/population combination were tested using ARLEQUIN version 3.1 (Schneider, Roessli, & Excoffier, 2000). Parameters of genetic variation were calculated using GenAlEx v6.41 (Peakall & Smouse, 2006), including the total number of alleles (Na), the effective number of alleles (Ne), the expected and observed heterozygosities (He and Ho, respectively), the Shannon information index (I) and the fixation (inbreeding) index (Fis). Additionally, FSTAT version 2.9.3.2 (Goudet, 2002) was used to calculate the allelic richness (AR), the unbiased estimate of Wright’s F-statistic (including total-population inbreeding coefficients (Fis)), the overall intrapopulation inbreeding coefficient (Fi) and the interpopulation genetic differentiation coefficient (Fst), Weir & Cockerham, 1984), and pairwise Fst between paired populations. Based on pairwise Fst, gene flow between populations (Nm) was further estimated with the following formula: Nm = (1 – Fst)/4Fst (Wright, 1969). Four abiotic-climate variables, namely, minimum temperature, maximum temperature, average temperature, and precipitation, from the sampled locations were obtained from the WorldClim database (Version 1.4; https://www.worldclim.org/) and used to calculate the differentiation matrix. Mantel tests (Mantel, 1967) between the matrix of the pairwise population differentiation in terms of Fst/(1 – Fst) and the differentiation matrix of geographic distances or abiotic-climate variables were performed with GenAlEx with 1,000 random permutations (Rousset, 1997).

Taking into account the geographic location of each population and the genetic differentiation within and among populations, Spatial Analysis of Molecular Variance (SAMOVA) software (Dupanloup, Schneider, & Excoffier, 2002) was used to define the best number of groups; then, ARLEQUIN version 3.11 was used for the analysis of molecular variance (AMOVA; Excoffier, Smouse, & Quattro, 1992), in which three levels of genetic differentiation were calculated: genetic differentiation within populations, genetic differentiation among populations within groups, and genetic differentiation among groups.

### TABLE 1 Groups based on the result from SAMOVA and geographic information for populations of Fokienia hodginsii

| Pop. ID | Geographic locality | Geographic coordinates | Altitude (m) | Sample size |
|---------|----------------------|------------------------|--------------|-------------|
| ZJJD    | Jiaoyi, Zhejiang, China | 119°33′19.98″E, 29°34′40.56″N | 877          | 20          |
| ZJFYS   | Longquan, Zhejiang, China | 119°10′11.05″E, 27°52′49.63″N | 1,471        | 20          |
| FJBL    | Nanjing, Fujian, China | 117°15′38.83″E, 24°31′13.57″N | 762          | 15          |
| FJDS    | Dehua, Fujian, China | 118°13′2.34″E, 25°38′27.1″N | 1,095        | 20          |
| FJHSA   | Shaxian, Fujian, China | 117°47′29.86″E, 26°23′32.6″N | 369          | 20          |
| FJMHS   | Longyan, Fujian, China | 116°51′17.78″E, 25°16′0.61″N | 830          | 20          |
| JXSQ    | Shangrao, Jiangxi, China | 118°3′50″E, 28°54′10.5″N | 1,354        | 20          |
| JXMTS   | Zixi, Jiangxi, China | 117°8′11.81″E, 27°50′6.31″N | 805          | 11          |
| GDQX    | Zhaoping, Guangdong, China | 111°57′56.82″E, 23°33′29.25″N | 1,068        | 20          |
| JXJGS   | Jinggangshan, Jiangxi, China | 114°09′16.36″E, 26°30′32.82″N | 1,311        | 20          |
| JXWSF   | Shangyou, Jiangxi, China | 114°19′12″E, 25°28′47.99″N | 1,488        | 20          |
| HNMS    | Yizhang, Hunan, China | 112°57′19.63″E, 24°57′49.43″N | 1,103        | 20          |
| HNVY    | Daoxian, Hunan, China | 111°20′45.39″E, 25°33′38.92″N | 1,247        | 23          |
| GXCWLS  | Baise, Guangxi, China | 106°22′36.07″E, 24°25′9.19″N | 1,671        | 20          |
| GXDMS   | Nanning, Guangxi, China | 108°26′17.47″E, 23°29′46.39″N | 1,203        | 5           |
| GXHP    | Longsheng, Guangxi, China | 109°54′51.55″E, 25°36′14.52″N | 1,290        | 20          |
| GXHJ    | Dongxing, Guangxi, China | 108°38′23.94″E, 25°12′9.82″N | 1,139        | 7           |
| GXJX    | Jinxiu, Guangxi, China | 110°19′15.11″E, 24°12′40.19″N | 989          | 20          |
| YNFLF   | Mengzi, Yunnan, China | 103°49′6.11″E, 22°52′12.27″N | 1,503        | 19          |
| GZYC    | Yucheng, Guizhou, China | 105°58′50.32″E, 27°22′2.01″N | 1,323        | 20          |
| CQSMS   | Jiangjin, Chongqing, China | 106°20′55.27″E, 28°34′38.61″N | 1,170        | 20          |
| SCHG    | Xuyong, Sichuan, China | 105°33′7.84″E, 28°14′40.64″N | 1,122        | 20          |
| V-PXB   | Fansipan, Sapa, Vietnam | 103°46′22.34″E, 22°21′03.54″N | 1,823        | 11          |
| V-HB    | Mai Châu, Hòa Bình, Vietnam | 104°53′25.10″E, 20°44′19.48″N | 1,366        | 16          |
BOTTLENECK 1.2.02 (Piry, Luikart, & Cornuet, 1999) was used to detect signals of recent bottleneck effects, in which one-tailed Wilcoxon signed-rank tests (10,000 replications) based on the “infinite allele model of mutation” (I.A.M.), the “stepwise mutation model” (S.M.M.), and the “two-phased model of mutation” (T.P.M.; 70% of alleles under S.M.M.) were performed, and Bonferroni corrections for multiple tests were made.

In addition, a Bayesian clustering approach implemented in STRUCTURE v2.3.4 (Evanno, Regnaut, & Goudet, 2005) was used to investigate population structure, in which a 100,000 burn-in period was followed by 10 iterations of 100,000 Markov chain Monte Carlo replicates per K (1–10). Then, STRUCTURE HARVESTER (Earl & Vonholdt, 2012) was used to determine the optimum K. Further, a principal coordinate analysis (PCoA) was conducted based on

![Figure 2](image_url)  
**Figure 2** Geographic locations of the 24 populations of *Fokienia hodginsii*

| Table 2 | The information for the 12 microsatellites |
|---------|------------------------------------------|
| Locus   | Primer sequences (5′–3′) | Repeat | Expected size (bp) | Putative function |
| F015    | F: TGTAATAACTCTGGTCCTTCC R: CTCTGTGCTCCTCCAA | (TA)7  | 200–210 | Arabidopsis thaliana SIT4 phosphatase-associated family protein |
| F017    | F: AAGACAAGATGCTCAGATCA R: GTGGTAGCCTAGAATTCTCAT | (AG)7  | 192–196 | Picea glauca clone GQ03325.106 mRNA |
| F020    | F: TTTCTGCTTGAATGAATCCA R: GCGGAGGAGAGGAGATT | (CT)7  | 232–238 | Arabidopsis thaliana armadillo/beta-catenin repeat family protein |
| F036    | F: GCGAGACAGAGATAGAGAA R: ATAGCATAACAGCCTCAT | (AG)6  | 260–268 | Oryza sativa (japonica cultivar-group) U1 small nuclear ribonucleoprotein 70 K |
| F042    | F: TGGAGAAGATAGGTCAAGG R: TCAATAGCTCCTCAGC | (GA)6  | 264–270 | Arabidopsis thaliana auxilin-like protein |
| F049    | F: CAATGTTCTCTGTGCTTG R: TTAGATCTGGAGTGGTGAAG | (CAG)7 | 221–245 | Picea sitchensis clone WS02761_D24 unknown mRNA |
| F089    | F: TACGATGAGCAGTCCTAT R: CACCTCCAACCACATTAC | (TGG)5  | 276–291 | Cryptomeria japonica putative glycine-rich RNA binding protein |
| F127    | F: CTTTCAACTTCTATAGGGAAGCAT R: TGACGCTCTACTGGAATG | (TTC)6  | 230–242 | Not found |
| F173    | F: TTATCTACAGGGCAAGCAT R: TATCTGGATAAGCAGGTGAG | (AAC)5  | 194–206 | Arabidopsis thaliana zinc-binding family protein |
| F204    | F: TCTGGGAATTTGGGGAAGR: CTGGCTCTATAAGGCTTAACTC | (CAG)5  | 201–210 | Pism sativum ultraviolet-B-repressible dehydrin-related protein |
| F210    | F: TGGAAGAAGAGAAGAGAGAAGATG R: CGGACCTCAGTGAAACTT | (GTG)5  | 291–306 | Not found |
| F217    | F: GCATATAGGTGCGGACTCR: GCAGAAGGTGCTGAGGAAG | (CAT)5  | 200–212 | Pinus radiata PrLTP1 |
### TABLE 3  Genetic variability for the 12 SSR markers within populations

| Pop   | N  | A_r | N_a | N_e | H_o  | H_e  | F_is | I   |
|-------|----|-----|-----|-----|------|------|------|-----|
| ZJJD  | 42 | 3.181 | 3.5 | 2.877 | 0.533 | 0.639 | 0.161 | 1.117 |
| ZJFYS | 45 | 3.365 | 3.75 | 3.089 | 0.496 | 0.659 | 0.241 | 1.178 |
| FJHBL | 44 | 3.348 | 3.667 | 3.024 | 0.544 | 0.666 | 0.18  | 1.178 |
| FJDYS | 43 | 3.318 | 3.583 | 3.106 | 0.521 | 0.669 | 0.22  | 1.179 |
| FJFHS | 43 | 3.273 | 3.583 | 3.031 | 0.542 | 0.658 | 0.166 | 1.158 |
| FJMHS | 44 | 3.283 | 3.667 | 2.989 | 0.517 | 0.658 | 0.21  | 1.161 |
| JXSQS | 43 | 3.253 | 3.583 | 3.062 | 0.567 | 0.656 | 0.124 | 1.152 |
| JXMTS | 39 | 3.078 | 3.25 | 2.804 | 0.583 | 0.628 | 0.062 | 1.069 |
| GDQXD | 41 | 3.2  |     |      |      |      |      |      |
| JXGS  | 40 | 3.083 | 3.333 | 2.775 | 0.563 | 0.624 | 0.09  | 1.077 |
| JXWZF | 41 | 3.114 | 3.417 | 2.804 | 0.521 | 0.63  | 0.175 | 1.091 |
| HNMS  | 42 | 3.251 | 3.5  | 3.023 | 0.563 | 0.662 | 0.147 | 1.156 |
| HNYY  | 42 | 3.147 | 3.5  | 2.826 | 0.496 | 0.634 | 0.219 | 1.106 |
| GXCWLS | 44 | 3.15 | 3.667 | 2.624 | 0.496 | 0.604 | 0.174 | 1.076 |
| GDMD  | 43 | 3.25 | 3.25 | 2.517 | 0.533 | 0.59  | 0.286 | 1.101 |
| GXHP  | 43 | 3.201 | 3.583 | 2.775 | 0.496 | 0.633 | 0.22  | 1.112 |
| GXHJ  | 39 | 3.147 | 3.25 | 2.662 | 0.524 | 0.606 | 0.262 | 1.04  |
| GXIX  | 44 | 3.244 | 3.667 | 3.048 | 0.475 | 0.661 | 0.084 | 1.159 |
| YNLFZ | 44 | 3.217 | 3.667 | 2.908 | 0.518 | 0.65  | 0.207 | 1.139 |
| GZYC  | 42 | 3.2  | 3.5  | 2.923 | 0.479 | 0.651 | 0.129 | 1.134 |
| CQMS  | 41 | 3.135 | 3.417 | 2.87  | 0.488 | 0.645 | 0.242 | 1.109 |
| SCHGX | 42 | 3.244 | 3.5 | 3.034 | 0.542 | 0.662 | 0.181 | 1.155 |
| V-PXB | 32 | 2.967 | 3   | 2.47  | 0.508 | 0.573 | 0.111 | 0.93  |
| V-HB  | 34 | 2.988 | 2.917 | 2.461 | 0.51  | 0.551 | 0.066 | 0.908 |
| Mean  |     | 3.193 ± 0.067 | 3.465 ± 0.044 | 2.861 ± 0.034 | 0.522 ± 0.007 | 0.635 ± 0.005 | 0.172 ± 0.011 | 1.105 ± 0.012 |

Notes. A_r: allelic richness; F_is: coefficient of inbreeding; H_o: expected frequency of heterozygotes; H_e: observed frequency of heterozygotes; I: Shannon index; N: number of alleles; N_r: observed number of alleles; N_e: effective number of alleles.

### TABLE 4  Genetic diversity at the 12 microsatellite loci

| Loci  | N_r | A_r | N_a | N_e | H_o  | H_e  | F_is | F_it | N_m |
|-------|-----|-----|-----|-----|------|------|------|------|-----|
| F015  | 8   | 4.233 | 4.000 | 3.405 | 0.583 | 0.700 | 0.167 | 0.284 | 0.140 | 1.533 |
| F017  | 6   | 2.769 | 2.958 | 2.609 | 0.541 | 0.607 | 0.109 | 0.227 | 0.132 | 1.647 |
| F020  | 5   | 4.071 | 3.250 | 2.846 | 0.429 | 0.636 | 0.326 | 0.411 | 0.126 | 1.730 |
| F036  | 9   | 3.323 | 4.292 | 3.294 | 0.522 | 0.688 | 0.241 | 0.342 | 0.133 | 1.634 |
| F042  | 4   | 3.520 | 3.917 | 3.213 | 0.552 | 0.686 | 0.195 | 0.216 | 0.025 | 9.610 |
| F049  | 7   | 3.214 | 2.875 | 2.415 | 0.546 | 0.574 | 0.048 | 0.334 | 0.300 | 0.582 |
| F089  | 5   | 3.399 | 3.000 | 2.415 | 0.531 | 0.548 | 0.065 | 0.250 | 0.198 | 1.012 |
| F127  | 7   | 4.546 | 4.375 | 3.433 | 0.574 | 0.699 | 0.178 | 0.283 | 0.127 | 1.712 |
| F137  | 7   | 3.926 | 3.125 | 2.452 | 0.407 | 0.589 | 0.308 | 0.431 | 0.178 | 1.158 |
| F204  | 6   | 3.279 | 2.958 | 2.637 | 0.518 | 0.616 | 0.158 | 0.304 | 0.173 | 1.194 |
| F210  | 8   | 3.947 | 3.625 | 2.721 | 0.520 | 0.617 | 0.156 | 0.323 | 0.198 | 1.016 |
| F217  | 6   | 4.171 | 3.208 | 2.890 | 0.541 | 0.644 | 0.161 | 0.293 | 0.158 | 1.330 |

Mean 3.695 ± 0.044 | 3.465 ± 0.044 | 2.861 ± 0.034 | 0.522 ± 0.007 | 0.635 ± 0.005 | 0.176 ± 0.024 | 0.308 ± 0.019 | 0.157 ± 0.019 | 2.013 ± 0.698 |

Notes. A_r: allelic richness, i.e. the average number of alleles per locus; F_is: inbreeding coefficient; F_it: total-population inbreeding coefficient; F_tr: among-population genetic differentiation coefficient; H_o: unbiased expected heterozygosity; H_e: observed heterozygosity; N_r: observed number of alleles; N_e: effective number of alleles; N_m: gene flow; N_r: number of alleles per locus.
the Jaccard distance between populations using MVSP software (Kovach, 1999).

3 | RESULTS

3.1 | Genetic diversity

According to the LD analysis for these 12 polymorphic loci, no pairs of loci showed linkage disequilibrium after a sequential Bonferroni correction for multiple tests, indicating that the 12 markers can be considered independent markers for population genetics studies. The genetic variation across the 24 natural populations is summarized in Table 3. According to Table 3, a total of 78 alleles were detected from these 12 SSR loci, ranging from 4 to 8 per locus. The average allelic richness \( (A_q) \) for each population ranged from 2.967 to 3.365 (average: 3.193 ± 0.067). The value of \( N_e \) ranged from 2.917 to 3.750 (average: 3.465 ± 0.044), \( N_s \) ranged from 2.461 to 3.106 (average: 2.861 ± 0.034), and \( H_e \) and \( H_o \) ranged from 0.551 to 0.669 (average: 0.635 ± 0.005) and 0.475 to 0.583 (average: 0.523 ± 0.007), respectively. After Bonferroni corrections, no loci showed deviations from Hardy–Weinberg equilibrium (Supporting Information Table S1).

The results from the LD analysis for these 12 polymorphic loci, no pairs of loci showed linkage disequilibrium after a sequential Bonferroni correction for multiple tests, indicating that the 12 markers can be considered independent markers for population genetics studies. The genetic variation across the 24 natural populations is summarized in Table 3. According to Table 3, a total of 78 alleles were detected from these 12 SSR loci, ranging from 4 to 8 per locus. The average allelic richness \( (A_q) \) for each population ranged from 2.967 to 3.365 (average: 3.193 ± 0.067). The value of \( N_e \) ranged from 2.917 to 3.750 (average: 3.465 ± 0.044), \( N_s \) ranged from 2.461 to 3.106 (average: 2.861 ± 0.034), and \( H_e \) and \( H_o \) ranged from 0.551 to 0.669 (average: 0.635 ± 0.005) and 0.475 to 0.583 (average: 0.523 ± 0.007), respectively. After Bonferroni corrections, no loci showed deviations from Hardy–Weinberg equilibrium (Supporting Information Table S1).

The \( F_{st} \) (inbreeding coefficient) averaged across all loci ranged from 0.048 to 0.326 (average: 0.176 ± 0.024, Table 4).

Populations V-PXB and V-HB, located in Vietnam, had the lowest genetic diversity (V-PXB: \( H_e = 0.573 \) and \( H_o = 0.508 \); V-HB: \( H_e = 0.551 \) and \( H_o = 0.510 \)). Among the 22 populations in China, GXDMS and GXHJ harbored the lowest genetic diversity (\( H_e = 0.590 \) and 0.606 and \( H_o = 0.533 \) and 0.524, respectively). In contrast, the populations FJDYS, FJHBL, HNMS and SCHGX showed the highest genetic diversity (\( H_e = 0.662-0.669 \) and \( H_o = 0.521 - 0.563 \)).

3.2 | Genetic structure

The results from \( F \)-statistics showed that the overall intrapopulation inbreeding coefficient \( (F_{in}) \) was 0.176 ± 0.024, the total-population inbreeding coefficient \( (F_{st}) \) was 0.308 ± 0.019, the interpopulation genetic differentiation coefficient \( (F_{is}) \) was 0.157 ± 0.019, and the gene flow \( (N_m) \) was estimated to be 2.013 ± 0.698 (Table 4). All pairwise \( F_{st} \) values were highly significant \( (p < 0.001) \), ranging from 0.009 (between FJDYS and FJFHS) to 0.234 (between V-HB and ZJJJD; Table 5). Correlation analyses showed that the genetic differentiation was most correlated with geographic distance \( (r = 0.882, p = 0.01) \), longitude changes \( (r = 0.466, p = 0.01) \), latitudinal changes \( (r = 0.432, p = 0.01) \), precipitation differentiation \( (r = 0.256, p = 0.01) \), elevational changes \( (r = 0.205, p = 0.01) \), and average temperature changes \( (r = 0.178, p = 0.04) \) (Table 6).

The SAMOVA demonstrated the highest value of \( F_{CT} \) \( (F_{CT} = 0.25346, p < 0.05) \) (Supporting Information Figure S1) when it divided all 24 populations into four groups as follows: the western China group including the populations located in western China (mostly the Yunnan–Guizhou Plateau); the central China group including the populations located in central China (Luoxiao Mountains, Nanling Mountains, and adjacent areas); the eastern China group including the populations located in central China (Luoxiao Mountains, Nanling Mountains, and adjacent areas); the eastern China group including the populations located in western China (Kovach, 1999).

4 | DISCUSSION

4.1 | Genetic diversity

Genetic diversity is crucial for species, as it may influence the ability of species to cope with environmental change (Frankham, Ballou, & Briscoe, 2002; Frankham, 1995a, 1995b). In this study, microsatellite markers were used to estimate population genetic diversity and to investigate the genetic structure of F. hodginsii. Slightly lower genetic diversity was found in F. hodginsii \( (H_e = 0.635 ± 0.005) \) than in Chamaecyparis obtusa \( (H_e = 0.780) \), the sister species of F. hodginsii (Matsumoto, Uchida, Taguchi, Tani, & Tsumura, 2010). Compared to other species (Ny bom, 2004), the expected heterozygosities \( (H_e) \) of F. hodginsii are similar to those of regional species \( (H_e = 0.65) \) and long-lived woody perennial species \( (H_e = 0.68) \). Allelic diversity \( (N_a) \) and expected heterozygosity \( (H_e) \) are also commonly used to estimate the genetic diversity in natural populations (Freeland,
### TABLE 5

| POP    | ZJJD | ZJYFS | FJHBL | FJDYS | FJFHS | FJMHS | JXMTS | JXSQS | JXJGS | JXWZF | GDQXD | HNMS | HNYY | GXJX | GXHP | GXHJ | GDXMS | GXCWLS | GZYC | CQSM | SCHG | YNLFZ | V-PXB | V-HB |
|--------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|------|------|------|------|-------|--------|------|------|------|-------|-------|------|
| ZJJD   | 0.000 | 15.728 | 16.894 | 8.984 | 7.049 | 9.803 | 8.197 | 4.429 | 2.827 | 2.753 | 3.140 |
| ZJYFS  | 0.016 | 0.000 | 13.977 | 10.007 | 8.882 | 10.419 | 6.643 | 3.796 | 2.555 | 2.571 | 2.627 |
| FJHBL  | 0.015 | 0.018 | 0.000 | 10.589 | 6.818 | 10.312 | 10.169 | 5.290 | 3.454 | 3.430 | 3.901 |
| FJDYS  | 0.027 | 0.024 | 0.023 | 0.000 | 29.065 | 22.317 | 5.358 | 4.917 | 3.143 | 3.288 | 3.337 |
| FJFHS  | 0.034 | 0.027 | 0.035 | 0.009 | 0.000 | 20.908 | 4.115 | 4.006 | 2.506 | 2.724 | 2.681 |
| FJMHS  | 0.025 | 0.023 | 0.024 | 0.011 | 0.012 | 0.000 | 4.969 | 4.514 | 2.763 | 2.887 | 3.113 |
| JXMTS  | 0.030 | 0.036 | 0.024 | 0.045 | 0.057 | 0.048 | 0.000 | 4.346 | 2.849 | 2.733 | 2.863 |
| JXSQS  | 0.053 | 0.062 | 0.045 | 0.048 | 0.059 | 0.052 | 0.054 | 0.000 | 7.558 | 8.447 | 8.834 |
| JXJGS  | 0.081 | 0.089 | 0.067 | 0.074 | 0.091 | 0.083 | 0.081 | 0.032 | 0.000 | 18.349 | 15.456 |
| JXWZF  | 0.083 | 0.089 | 0.068 | 0.071 | 0.084 | 0.080 | 0.084 | 0.029 | 0.013 | 0.000 | 15.694 |
| GDQXD  | 0.074 | 0.087 | 0.060 | 0.070 | 0.085 | 0.074 | 0.080 | 0.028 | 0.016 | 0.016 | 0.000 |
| HNMS   | 0.070 | 0.075 | 0.066 | 0.065 | 0.076 | 0.073 | 0.095 | 0.037 | 0.028 | 0.025 | 0.028 |
| HNYY   | 0.078 | 0.083 | 0.072 | 0.081 | 0.092 | 0.083 | 0.098 | 0.045 | 0.026 | 0.025 | 0.025 |
| GXJX   | 0.084 | 0.087 | 0.071 | 0.076 | 0.086 | 0.080 | 0.092 | 0.044 | 0.044 | 0.034 | 0.042 |
| GXHP   | 0.094 | 0.097 | 0.082 | 0.078 | 0.087 | 0.077 | 0.117 | 0.063 | 0.063 | 0.048 | 0.052 |
| GXHJ   | 0.115 | 0.123 | 0.109 | 0.105 | 0.114 | 0.106 | 0.129 | 0.089 | 0.088 | 0.072 | 0.072 |
| GDXMS  | 0.095 | 0.100 | 0.084 | 0.079 | 0.087 | 0.076 | 0.116 | 0.083 | 0.084 | 0.072 | 0.067 |
| GXCWLS | 0.101 | 0.112 | 0.094 | 0.087 | 0.095 | 0.084 | 0.124 | 0.070 | 0.075 | 0.056 | 0.057 |
| GZYC   | 0.093 | 0.098 | 0.082 | 0.085 | 0.097 | 0.087 | 0.099 | 0.078 | 0.075 | 0.067 | 0.072 |
| CQSM   | 0.093 | 0.099 | 0.085 | 0.087 | 0.099 | 0.089 | 0.107 | 0.082 | 0.073 | 0.069 | 0.076 |
| SCHG   | 0.093 | 0.096 | 0.085 | 0.091 | 0.100 | 0.094 | 0.101 | 0.080 | 0.069 | 0.066 | 0.073 |
| YNLFZ  | 0.099 | 0.104 | 0.093 | 0.092 | 0.102 | 0.095 | 0.113 | 0.085 | 0.083 | 0.073 | 0.078 |
| V-PXB  | 0.196 | 0.178 | 0.179 | 0.183 | 0.187 | 0.191 | 0.185 | 0.165 | 0.189 | 0.183 | 0.186 |
| V-HB   | 0.234 | 0.211 | 0.203 | 0.211 | 0.217 | 0.216 | 0.213 | 0.198 | 0.209 | 0.204 | 0.213 |

Kirk, & Petersen, 2011; Hamilton, 2009). The \( H_e \) and \( N_a \) values of *F. hodginsii* \( (H_e = 0.635, N_a = 3.465) \) are slightly lower than those of *C. obtusa* (\( H_e = 0.780, N_a = 7.038 \)), albeit higher than those of other conifer species, such as Cryptomeria japonica (\( H_e = 0.277, N_a = 2.000 \), Tsumura & Tomaru, 1999).

In this study, the lowest genetic diversity was found in the two populations in Vietnam (V-PXB: \( H_e = 0.573 \); V-HB: \( H_e = 0.551 \)). This phenomenon agreed with previous reports that most populations in Vietnam harbor low genetic diversity (\( H_e = 0.0970 \pm 0.0101 \), ISSR markers used by Tam et al., 2011). It is possible that China serves as the central distributional area of *F. hodginsii*, such that its genetic diversity decreased as it dispersed from its central area to its marginal areas, such as Vietnam (Wei, Sork, Meng, & Jiang, 2016). Tam et al. (2011) also indicated that, as a result of human disturbance, the *F. hodginsii* habitat in Vietnam has been degraded and fragmented, which may also serve as a good explanation for the low genetic variability in Vietnam, as signals of bottleneck events were also detected in these two populations.

**FIGURE 3** Relationship between pairwise \( F_{st}/(1 - F_{st}) \) and the geographic distance among the populations of *Fokienia hodginsii* \( (r = 0.882, p = 0.01) \)
In China, the populations GXDMS and GXHJ, where only 5–7 individuals were collected, had the lowest genetic diversity ($H_e = 0.590$ and 0.606, respectively), and signals of bottleneck events were also detected in these two populations (Table 8). These phenomena may be explained by insufficient sampling. However, as a Tertiary relict species, this conifer was strongly influenced by the Pleistocene glaciers, resulting in the populations contracting sharply. In China, it has been more than 2,600 years since this conifer was used to build boats and houses, and due to extensive deforestation, the lower distribution limit of this conifer has moved up by 500 m since the 1980s (Hou, Cheng, Lin, & Yu, 2004). During our field investigations, we also observed substantial evidence of deforestation near the F. hodginsii populations, and in many places where ample specimens were recorded, few or no individual were found, especially in the populations of GXDMS and GXHJ. Further, the geographic locations of these two populations were near Vietnam, indicating that the low genetic diversity observed in GXDMS and GXHJ may be caused by the same factors that account for the low genetic diversity observed in Vietnam.

TABLE 6  The relationship between genetic differentiation ($F_{ST}$) and the differences in environmental factors

| Influencing factors | Formula | $r$ | $p$ |
|---------------------|---------|-----|-----|
| $\Delta_{\text{max}}$ temperature | $y = 0.0015x + 0.0808$ | 0.067 | 0.27 |
| $\Delta_{\text{average}}$ temperature | $y = 0.0017x + 0.0798$ | 0.178 | 0.04 |
| $\Delta_{\text{max}}$ precipitation | $y = 0.0019x + 0.0786$ | 0.092 | 0.21 |
| $\Delta_{\text{precipitation}}$ | $y = 4E-05x + 0.0676$ | 0.256 | 0.01 |
| $\Delta_{\text{elevation}}$ | $y = 3E-05x + 0.00707$ | 0.205 | 0.1 |
| $\Delta_{\text{latitude}}$ | $y = 0.0094x + 0.052$ | 0.432 | 0.01 |
| $\Delta_{\text{longitude}}$ | $y = 0.0043x + 0.0478$ | 0.466 | 0.01 |

TABLE 7  Analysis of molecular variance (AMOVA) for the 24 populations

| Source of variation | Sum of squares | Variance components | Percentage of variation | F-statistics |
|---------------------|----------------|---------------------|------------------------|--------------|
| Among groups        | 394.651        | 0.61683             | 13.14                  | $F_{CT}=0.21430$ |
| Among populations within groups | 169.975 | 0.10347             | 2.20                   | $F_{ST}=0.02538$ |
| Within populations  | 3277.343       | 3.97323             | 84.66                  | $F_{CT}=0.13142$ |
| Total               | 3841.969       | 4.69353             | 100.00                 |              |
4.2 Genetic differentiation

Most conifers have high levels of genetic diversity within populations and low levels of differentiation among populations (Hamrick, Godt, & Sherman-Broyles, 1992). According to the AMOVA results in this study, the genetic diversity of *F. hodginsii* is primarily maintained within populations (84.66%, *p* < 0.01), while the genetic differentiation among populations of *F. hodginsii* (*F_{ST} = 0.157 ± 0.019*) is weak; however, the value of *F_{IS}* was 0.176 ± 0.024, indicating a mixed mating system in which inbreeding occurred frequently. The genetic differentiation among populations of *F. hodginsii* (*F_{ST} = 0.157 ± 0.019*) is also in accordance with that of other mixed-breeding species of seed plants (79.2%, Nybom & Bartish, 2000), slightly higher than that of wind-dispersed species (*F_{ST} = 0.13*), and much lower than that of entomophilous species (*F_{ST} = 0.21*) (Nybom, 2004). This pattern is also in accordance with previous observations that the dispersal of *Fokienia* is mainly through the wind, though sometimes also through insects (Jin et al., 2012; Lu et al., 2011; Wang & Ran, 2014). Such patterns were also observed in *Cupressus funebris*, for which the genetic diversity within populations is 88.15%, *F_{ST} = 0.1580* and *F_{IS} = 0.1579* (Lu et al., 2014). For the species *C. obtusa*, much higher genetic diversity was maintained within populations (91.7%), and genetic differentiation among populations was lower (*F_{ST} = 0.039*). The *F_{IS}* value estimated for *C. obtusa* was only 0.034, indicating a random mating system. Therefore, the different levels of genetic differentiation among the three species may be caused primarily by the differentiation of mating systems.

In this study, a significant correlation was found between genetic differentiation (*F_{ST}/(1 − F_{ST})*) and geographic distance (*r* = 0.882, *p* = 0.01), suggesting that the genetic differentiation among populations follows the model of isolation by distance (IBD), that is, the differentiation among populations is strongly associated with geographic distance. Such a phenomenon was also observed in *C. obtusa* (*r^2 = 0.3997* and *p = 0.001*, Matsumoto et al., 2010). It is also known that the dispersal of *Fokienia* is mainly through the wind (Jin et al., 2012; Lu et al., 2011; Wang & Ran, 2014); thus, its capability for long-distance dispersal could be limited as the geographic distance increases.

Although significant correlations were also found between genetic differentiation and climatic variables in the sampled locations,
FIGURE 6  Grouping of populations according to STRUCTURE ($K = 3$ or $K = 4$) and their geographic locations.

FIGURE 7  Principal coordinate analysis of individual genotypes obtained from four groups.
such as average temperature ($r = 0.178$, $p = 0.04$) and precipitation ($r = 0.256$, $p = 0.01$), their correlations were rather weak compared to those with geographic distance ($r = 0.882$, $p = 0.01$). It was observed that the flowering period of *F. hodginsi* is delayed with a decrease in temperature and precipitation (Hou et al., 2006); therefore, climatic factors may also actively increase the genetic differentiation among populations to a lesser extent.

### 4.3 Population structure

The STRUCTURE model based on 12 loci identified three as the most likely number of genetic clusters, as the highest $\Delta K$ value was at $K = 3$. The assignment results for $K = 3$ showed that the two populations in Vietnam were clustered with the eastern China group. In contrast, the results for $K = 4$ showed that the Vietnam populations were separated from the eastern China group and clustered as a fourth group. However, the populations located in Vietnam are located far away from those in eastern China, and the climatic conditions are much different between the two regions. It is surprising that the two populations in Vietnam were clustered with the eastern China group and not the western China group, which is much closer to Vietnam in terms of geographic distance. More molecular data need to be analyzed to understand this pattern.

In this study, the assignment results for $K = 4$ were the same as the results from SAMOVA and PCoA. Therefore, it is reasonable to divide all populations into four groups: the eastern China group, the central China group, the western China group, and the Vietnam group. The terrain of China from west to east forms a flight of three steps, commonly called the “Three Steps”. The first step located in southwestern China mainly includes the Qinghai-Tibetan Plateau, which has an elevation above 4,000 m. The second step lies in central and western China with an elevation of 1,000–3,000 m and includes the Xuefeng Mountains, Qinling Mountains, and Yunnan–Guizhou Plateau. The third step spans all remaining regions, covering eastern and southern China with an elevation of 500 m (Huang et al., 2012). The western China group is located on the second step, which mainly contains plateau and basin, while the central China group and the eastern China group are located on the third step, which mainly contains plain and hills. Additionally, the elevation of the sampled populations in the western China group is generally higher than that of populations in the central China group and eastern China group (Table 1). According to Hou et al. (2006), the flowering period of

| POP ID | Wilcoxon test | Sign test | Model shift test |
|--------|--------------|-----------|-----------------|
|        | I.A.M. | T.P.M. | I.A.M. | T.P.M. |                     |
| ZJJD   | 0.0744 | 0.1618 | 0.2645 | 0.0623 | L-shaped            |
| ZJYFS  | 0.0853 | 0.1543 | 0.4768 | 0.1857 | L-shaped            |
| FJHBL  | 0.1034 | 0.1764 | 0.3783 | 0.2879 | L-shaped            |
| FJDYS  | 0.0953 | 0.1665 | 0.0624 | 0.2645 | L-shaped            |
| FJFHS  | 0.0847 | 0.1555 | 0.1742 | 0.6829 | L-shaped            |
| FJMHPS | 0.0963 | 0.1685 | 0.5305 | 0.1198 | L-shaped            |
| JXSQS  | 0.0748 | 0.133  | 0.3195 | 0.0456 | L-shaped            |
| JXMSTS | 0.0764 | 0.1319 | 0.381  | 0.2663 | L-shaped            |
| GDQXD  | 0.0608 | 0.1338 | 0.5969 | 0.6244 | L-shaped            |
| JXJGS  | 0.0608 | 0.1219 | 0.3142 | 0.2091 | L-shaped            |
| JXWZF  | 0.0543 | 0.1256 | 0.3201 | 0.3694 | L-shaped            |
| HNMS   | 0.0814 | 0.1706 | 0.3142 | 0.2377 | L-shaped            |
| HNYY   | 0.0764 | 0.1391 | 0.12  | 0.1542 | L-shaped            |
| GXCWLS | 0.0975 | 0.625  | 0.1857 | 0.2645 | L-shaped            |
| GXDMS  | 0.0159 | 0.0312 | 0.0288 | 0.048  | L-shaped            |
| GXHP   | 0.1019 | 0.1497 | 0.6829 | 0.6238 | L-shaped            |
| GXHX   | 0.0858 | 0.1531 | 0.1238 | 0.1742 | L-shaped            |
| YNLFZ  | 0.0921 | 0.16    | 0.4487 | 0.5305 | L-shaped            |
| GZYC   | 0.0715 | 0.1479 | 0.2397 | 0.3192 | L-shaped            |
| CQSMS  | 0.091  | 0.1624 | 0.0803 | 0.3711 | L-shaped            |
| SCHX   | 0.0784 | 0.1574 | 0.3169 | 0.4143 | L-shaped            |
| V-PXB  | 0.0472 | 0.0264 | 0.0278 | 0.0326 | L-shaped            |
| V-HB   | 0.0376 | 0.0473 | 0.0154 | 0.0471 | L-shaped            |

Note. I.A.M.: infinite allele model of mutation; T.P.M.: two-phased model of mutation. The bold values represent the significance values lower than 0.05 ($p < 0.05$).
F. hodginsii is delayed with an increase in elevation. Therefore, the change in topography may be the main reason for the population differentiation between the western China group and the central China group. Based on the specimen records and our field collections, the distribution of F. hodginsii is continuous between the western China group and the central China group; thus, populations located near the border, such as GXJX and GXXM, may receive gene flow from both groups and ultimately harbor mixed gene pools.

Population differentiation was also found between the central China group and the eastern China group even though both of them are located on the third step. It was found that the central China group belongs to the Guangdong and Guangxi Hills while the eastern China group belongs to the Zhejiang and Fujian Hills, and between them, most areas are plains with a low elevation where no specimen records of F. hodginsii were found. Therefore, the plain area between the central and eastern China groups may have limited the gene flow between them and led to genetic differentiation, as we have found that isolation by distance was the main reason for genetic differentiation of F. hodginsii. However, it was surprisingly that the population JXSQS, located in the eastern China group, was closer to the central China group genetically (Figure 5). It is possible that some of the individuals could be later generations of ancient transplants from the central area, considering that F. hodginsii was often planted around the tombs and temples in China.

4.4 | Conservation implications

Genetic diversity plays an important role in determining the survival and adaptability of a species (Liao et al., 2015). The high genetic diversity maintained within F. hodginsii and the initial significant genetic differentiation among its populations found in this study are encouraging. However, we found recent bottleneck events in the populations GXDMS, GXHJ, V-PXB, and V-HB, suggesting that individual populations may suffer from a dramatic decline in population size. As a Tertiary relict species, the range of this conifer contracted sharply during the Pleistocene glaciations, and our field investigations also showed that the F. hodginsii populations have been overexploited since the 1980s, especially in the last ten years. For the conservation of this species, measures should be taken to increase the number of individuals and avoid the destruction caused by human activities. In situ conservation and breeding can also be considered to maintain the greatest within-species genetic variation, especially for the populations GXHJ and GXDMS, with higher inbreeding coefficients. Establishing seed orchards is also a good method, which could preserve favorable genes and prepare for breeding in the future. According to the results from STRUCTURE, the optimum number of groups is 4; thus, we also should establish seed orchards for these four groups to preserve their genotypes. In addition, establishing multiple F. hodginsii nature reserves, such as the Daiyunshan National Nature Reserve and Nanling National Nature Reserve, is needed, and the communities containing F. hodginsii should be classified as absolute protection areas to avoid human destruction.

ACKNOWLEDGMENTS

We thank the Daiyunshan National Nature Reserve, Mangshan National Nature Reserve, and Vietnam National Museum of Nature for allowing us to collect samples. This work was supported by the National Natural Science Foundation of China (31670189 and 31570195), the Natural Science Foundation of Guangdong Province, China(2016A030313326), the Special Program for Science and Technology Basic Research of the Ministry of Science and Technology of China (2013FY111500), the Foundation of Jingtangshan Administration of Jiangxi Province (33000-7102993), the Fourth National Survey on Chinese Material Medical Resources Program for State Administration of Traditional Chinese Medicine of the People’s Republic of China (2017-152-003), the Fundamental Research Funds for the Central Universities (16lgjc38), and the Chang Hungta Science Foundation of Sun Yat-sen University.

CONFLICT OF INTEREST

None declared.

AUTHORS’ CONTRIBUTIONS

Liao, W.B. and Fan, Q. designed the research. Guo, W. and Huang, Y.SH. collected the samples. Yin, Q.Y., Huang, Y.L. and Zhou, R.CH. generated the data. Yin, Q.Y., Chen, S.F. and Zhou, R.CH. analyzed and interpreted the data. Yin, Q.Y. wrote the manuscript, and Chen, S.F. and Zhou, R.CH. edited the manuscript.

DATA ACCESSIBILITY

The primers used in this study are shown in Table 2, and all other data supporting the findings are available within the article and supplementary information file.

ORCID

Qiang Fan http://orcid.org/0000-0003-4254-6936

REFERENCES

Caughley, G. (1994). Directions in conservation biology. Journal of Animal Ecology, 63(2), 215–244. https://doi.org/10.2307/5542

Ding, M. Y., Meng, K. K., Fan, Q., Tan, W. Z., Liao, W. B., & Chen, S. F. (2017). Development and validation of EST-SSR markers for Fokienia hodginsii (Cupressaceae). Applications in Plant Sciences, 5(3), 1600152. https://doi.org/10.3732/apps.1600152.

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19(1), 11-15. https://doi.org/10.2307/4119796

Dupanloup, I., Schneider, S., & Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. Molecular Ecology, 11(12), 2571. https://doi.org/10.1046/j.1365-294X.2002.01650.x

Earl, D. A., & Vonholdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and
implementing the Evanno method. Conservation Genetics Resources, 4(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species’ geographical ranges: The central–marginal hypothesis and beyond. Molecular Ecology, 17(5), 1170–1188. https://doi.org/10.1111/j.1365-294X.200703659.x

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology, 14(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial-DNA restriction data. Genetics, 131(2), 479–491.

Farjon, A. (2005). A monograph of Cupressaceae and Sciadopitys. Richmond, Surrey: Royal Botanic Gardens.

Frankham, R. (1995a). Conservation genetics. Oxford, UK: John Wiley & Sons.

Frankham, R. (1995b). Inbreeding and extinction: A threshold effect. Conservation Biology, 9(4), 792–799. https:// doi.org/10.1046/j.1523-1739.1995.09040792.x

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2002). Introduction to conservation genetics (p. 617). Cambridge, UK: Cambridge University Press.

Freeland, J. R., Kirk, H., & Petersen, S. (2011). Genetic variation and fixation indices. Molecular Ecology, 2nd ed. Chichester, UK: John Wiley & Sons.

Goudet, J. (2002).

Guo, S. X., & Zhang, G. F. (2002). Oligocene Sanhe flora in Longjing County of Jilin, Northeast China. Acta Palaeontologica Sinica, 41(2), 193–210.

Hamilton, B. M., ed. (2009). Molecular evolution. In Population genetics (pp. 235–282). Oxford, UK: Wiley-Blackwell.

Hamrick, J. L., Godt, M. J. W., & Sherman-Broyles, S. L. (1992). Factors influencing levels of genetic diversity in woody plant species. New Forests, 6(1–4), 95–124. https://doi.org/10.1007/978-94-011-2815-5_7

He, W., Sun, B. N., & Liu, Y. S. (2012). Fokienia shengxianensis sp nov (Cupressaceae) from the late Miocene of eastern China and its paleoecological implications. Review of Palaeobotany and Palynology, 176–177(1), 24–34. https://doi.org/10.1016/j.revpalbo.2012.03.013

Hou, B., Cheng, Z. H., Lin, F., & Yu, G. (2004). Historical development of the name of Fokienia hodginsii. Human Forestry Science & Technology, 31(3), 68–71.

Hou, B., Lin, F., Yu, G., Cheng, Z., Zhang, X., & Tao, S. (2006). Study on phenology of flower and cone of Fokienia hodginsii. Chinese Wild Plant Resources, 25(1), 45–47.

Huang, J., Chen, B., Liu, C., Lai, J., Zhang, J., & Ma, K. (2012). Identifying hotspots of endemic woody seed plant diversity in China. Diversity and Distributions, 18(7), 673–688. https://doi.org/10.1111/j.1472-4642.2011.00845.x

Huang, S., Huang, L., Guo, S., & Zheng, Y. (2015). Investigation of natural resources of fokienia and some suggestions. Journal of Green Science and Technology, 3(3), 145–146.

Huang, S. J., Rong, J. D., Zhang, L. H., Yang, Y., Jiang, J. L., & Zheng, Y. S. (2013). Research summarization of Fokienia hodgirtisii. Journal of Fujian Forestry Science and Technology, 8(5), 319–319.

Jin, B., Tang, L., Yu, W., Wang, D., Zhang, M., & Ma, J. X. (2012). Temporal and spatial characteristics of male cone development in Metasequoia glyptostroboides Hu et Cheng. Plant Signaling and Behavior, 7(12), 1687–1694. https://doi.org/10.4161/psb.22898

Kovach, W. L. (1999). MVSP – A MultiVariate statistical package for windows, ver. 3.1 (p. 133). Pentraeth, Wales, Great Britain: Kovach Computing Services.

Lande, R. (1993). Risks of population extinction from demographic and environmental stochasticity and random catastrophes. American Naturalist, 142(6), 911–927. https://doi.org/10.1086/285580

Lesica, P., & Allendorf, F. W. (2010). When are peripheral populations valuable for conservation? Conservation Biology, 9(4), 753–760. https://doi.org/10.1111/j.1523-1739.1995.09040792.x

Liao, S. C., Kui, K., Tian, B., Zhang, Z., Liu, A., Li, K., & Lang, X. (2015). The effect of long-term historical habitat fragmentation on genetic diversity of the relictual conifer Calocedrus macrolepis (Cupressaceae) in china. Brazilian Journal of Botany, 38(3), 567–577. https://doi.org/10.1016/j.bjbp.2015.01-0168-4

Lu, Y., Jin, B., Wang, L., Wang, Y., Dong, J., Jiang, X. X., & Chen, P. (2011). Adaptation of male reproductive structures to wind pollination in gymnosperms: Cones and pollen grains. Canadian Journal of Plant Science, 91(5), 897–906. https://doi.org/10.4141/cjps-2011-020

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. Cancer Research, 27(2), 209–220.

Matsumoto, A., Uchida, K., Taguchi, Y., Tani, N., & Tsumura, Y. (2010). Genetic diversity and structure of natural fragmented Chamaecyparis obtusa populations as revealed by microsatellite markers. Journal of Plant Research, 123(5), 689–699. https://doi.org/10.1007/s10265-009-0299-4

Mclver, E. E., & Basinger, J. F. (1990). Fossil seed cones of Fokienia (Cupressaceae) from the paleocene ravenscrag formation of saskatchewan, Canada. Canadian Journal of Botany-revue Canadienne De Botanique, 68(7), 1609–1618. https://doi.org/10.1139/b90-207

Nyblom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology, 13(5), 1143–1155. https://doi.org/10.1111/j.1365-294X.2004.02141.x

Nyblom, H., & Bartish, I. V. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in Plant Evolution and Systematics, 3(2), 93–114. https://doi.org/10.1074/ajb.2001.00006

Peakall, R., & Smouse, P. E. (2006). Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Resources, 6(1), 288–295. https://doi.org/10.1111/j.1471-8296.2005.01155.x

Piry, S. G., Luikart, G. L., & Cornuet, J. M. (1999). BOTTLENECK: A computer program for detecting recent reductions in effective population size using allele frequency data. Journal of Heredity, 90(4), 502–503. https://doi.org/10.1093/oxfordjournals.jhered.a045502

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by Distance. Genetics, 145(4), 1219–1228.

Schneider, S., Roessli, D., & Excoffier, L. (2000). Arlequin, Version 2000: A Software for Population Genetics Data Analysis. Geneva, Switzerland: University of Geneva.

Tam, M. N., Trang, N., & Hoa, N. T. (2011). Genetic diversity of an endangered species, Fokienia hodginsii (Cupressaceae). African Journal of Biotechnology, 10(71), 15838–15844. https://doi.org/10.5897/AJB10.2299

Tsumura, Y., & Tomaru, N. (1999). Genetic diversity of Cryptomeria japonica using co-dominant DNA markers based on sequenced-tagged sites. Theoretical and Applied Genetics, 98(4), 396–404. https://doi.org/10.1007/s001220051085

Vuong, D. H. (2009). Studies on Fokienia hodginsii community in Vietnam Hoanglien National Park. Journal of Anhui. Agricultural Sciences, 37(9), 4024–4028, 4031.

Wang, X. Q., Huang, Y., & Long, C. L. (2013). Assessing the genetic consequences of flower-harvesting in Rhododendron decorum Franchet (Ericaceae) using microsatellite markers. Biochemical
Systematics and Ecology, 50(10), 296–303. https://doi.org/10.1016/j.bse.2013.04.009
Wang, X. Q., & Ran, J. H. (2014). Evolution and biogeography of gymnosperms. Molecular Phylogenetics and Evolution, 75(1), 24–40. https://doi.org/10.1016/j.ympev.2013.04.005
Wei, X., Sork, V. L., Meng, H., & Jiang, M. (2016). Genetic evidence for central-marginal hypothesis in a Cenozoic relict tree species across its distribution in China. Journal of Biogeography, 43(11), 2173–2185. https://doi.org/10.1111/jbi.12788.
Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the analysis of population structure. Evolution, 38(6), 1358–1370. https://doi.org/10.2307/2408641
Wright, S. (1969). Evolution and Genetics of Populations, Vol. 2. The Theory of Gene Frequencies. Chicago, IL: University of Chicago Press.
Yang, Z. Y., Ran, J. H., & Wang, X. Q. (2012). Tree genome-based phylogeny of Cupressaceae: Further evidence for the evolution of gymnosperms and Southern Hemisphere biogeography. Molecular Phylogenetics and Evolution, 64(3), 452–470. https://doi.org/10.1016/j.ympev.2012.05.004
Zhao, Q. Y. (2005). A review of the advance in forestation and utilization of Fokienia hodginsii. Subtropical Plant Science, 34(3), 78–81.
Zheng, W. J., & Fu, G. L. (1978). Flora reipublicae popularis sinicae, Tomus 7 (pp. 313–398). Beijing, China: Science Press.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Yin Q, Chen S, Guo W, et al. Pronounced genetic differentiation in Fokienia hodginsii revealed by simple sequence repeat markers. Ecol Evol. 2018;8:10938–10951. https://doi.org/10.1002/ece3.4560