Genetic Diversity and Genetic Structure of Three Camellia Species Based on SSR Markers: A Post-print

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Date: 2018-05-07T00:00:00+00:00

Abstract

Genetic diversity is intimately associated with a species' adaptive capacity and evolutionary potential. Understanding the genetic diversity and genetic structure of rare and endangered plants can provide a theoretical foundation for developing conservation strategies and management protocols. Camellia chrysanthoides, Camellia micrantha, and Camellia parvipetala represent three endangered Camellia species. This study employed microsatellite markers to analyze genetic diversity and genetic structure across 7 populations comprising 184 individuals. A total of 92 alleles were detected at 11 loci. At the species level, Camellia parvipetala exhibited a mean number of alleles (NA) of 3.9, effective number of alleles (NE) of 2.328, observed heterozygosity (Ho) of 0.520, and expected heterozygosity (He) of 0.501, which were higher than those of Camellia chrysanthoides and Camellia micrantha. At the population level, the effective number of alleles (NE) ranged from 1.788 to 2.466, and expected heterozygosity (He) ranged from 0.379 to 0.543; the coefficient of genetic differentiation among populations (FST) ranged from 0.1437 to 0.4533, and gene flow among populations (Nm) ranged from 0.3015 to 1.4889. AMOVA molecular variance analysis revealed that 65.72% of the variation resided within populations. The three Camellia species exhibited low levels of genetic diversity and high levels of genetic differentiation among populations. STRUCTURE and PCoA analyses of population genetic structure partitioned the sampled populations into two groups, with most individuals of Camellia chrysanthoides and Camellia micrantha clustering in one group, and most individuals of Camellia parvipetala clustering in another group. All extant populations should implement in situ or ex situ conservation measures as soon as possible according to specific circumstances.

Full Text

Preamble

Genetic Diversity and Structure of Three Yellow Camellia Species Based on SSR Markers

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DOI: 10.11931/guihaia.gxzw201803045

Abstract

Genetic diversity is closely associated with species' adaptability and evolutionary potential. Understanding the genetic diversity and structure of rare and endangered plants provides a theoretical foundation for developing conservation strategies and management approaches. *Camellia chrysanthoides*, *C. micrantha*, and *C. parvipetala* are three endangered yellow camellia species. This study used microsatellite markers to analyze genetic diversity and population structure across 184 individuals from seven populations. A total of 92 alleles were detected at 11 loci. At the species level, *C. parvipetala* exhibited higher values than the other two species for mean number of alleles (NA = 3.9), effective number of alleles (NE = 2.328), observed heterozygosity (Ho = 0.520), and expected heterozygosity (He = 0.501). At the population level, the effective number of alleles ranged from 1.788 to 2.466, and expected heterozygosity ranged from 0.379 to 0.543. Pairwise genetic differentiation coefficients (FST) ranged from 0.1437 to 0.4533, while gene flow (Nm) ranged from 0.3015 to 1.4889. AMOVA analysis revealed that 65.72% of variation occurred within populations. These three camellia species showed low genetic diversity and high genetic differentiation among populations. STRUCTURE and PCoA analyses grouped the sampled populations into two clusters: most individuals of *C. chrysanthoides* and *C. micrantha* formed one group, while most *C. parvipetala* individuals formed another. All extant populations should receive either in situ or ex situ conservation measures as soon as possible based on their actual conditions.

Keywords: *Camellia chrysanthoides*; *Camellia micrantha*; *Camellia parvipetala*; genetic diversity; genetic structure

Funding: National Natural Science Foundation of China (31260053); Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection, Ministry of Education (ERESEP2015Z01); Innovation Project of Guangxi

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1. Introduction

Camellia chrysanthoides H. T. Chang (2n=30), *Camellia micrantha* S. Y. Liang et Y. C. Zhong (2n=30), and *Camellia parvipetala* J. Y. Liang et Su (2n=30) are three yellow camellia species distributed in southwestern Guangxi, China (Zhang and Ren, 1998; Liang, 1995). *Camellia chrysanthoides* occurs in Daqingshan, Longzhou County; *C. micrantha* in Xiashi Town, Pingxiang City; and *C. parvipetala* in Ningming County. Their distribution ranges are extremely narrow and geographically proximate. Habitat destruction from land development and utilization, combined with transplantation of wild plants by local residents for their ornamental value, has caused rapid population decline and fragmented distribution in these three species. Both *C. chrysanthoides* and *C. micrantha* have been listed as endangered in the *Threatened Species List of China*' *s Higher Plants* (Qin et al., 2017).

Genetic diversity represents a species' adaptive capacity and evolutionary potential, enabling it to cope with environmental changes (Frankham et al., 2002). Studies on genetic diversity and structure in rare and endangered plants not only reveal the mechanisms leading to endangerment but also provide theoretical foundations for conservation strategies and management (Segarra-Moragues et al., 2005; Su et al., 2017). Molecular markers have become a common method for estimating genetic variation and structure in endangered species (Ryall, 1998). Microsatellites, also known as simple sequence repeats (SSRs), offer advantages including high polymorphism, codominance, stability, good reproducibility, and widespread occurrence in eukaryotes, making them effective tools for plant population genetics research (Li et al., 2013; György et al., 2014; Meng et al., 2015). SSR markers have been widely applied to reveal genetic diversity and structure in endangered plant populations (Yang et al., 2016; Ma et al., 2015; Zhao et al., 2017; He et al., 2017).

Genetic diversity analysis serves as an important indicator for evaluating and conserving endangered plants, providing crucial information for developing effective conservation measures (Cires et al., 2011). No previous studies have reported on the genetic diversity and structure of *C. chrysanthoides*, *C. micrantha*, and *C. parvipetala*. Therefore, this study used microsatellite markers to assess genetic diversity and structure in seven populations of these three

camellia species, aiming to understand their genetic characteristics and propose conservation strategies based on the findings.

2.1 Materials

No taxonomic controversies exist regarding *C. chrysanthoides* and *C. micrantha* (Zhang and Ren, 1998; Ming and Bartholomew, 2007). Although Ming and Bartholomew (2007) synonymized *C. parvipetala* with *C. indochinensis* Merrill, morphological differences exist between them. Therefore, we followed Zhang and Ren (1998) and treated *C. parvipetala* as an independent species for sampling. We located seven populations across the entire distribution range. The distribution and morphological characteristics of three *C. micrantha* populations and two *C. parvipetala* populations matched the original descriptions. Populations BH1 and BH2 exhibited maximum flower diameters of only 3 cm, which does not match the 4–5.5 cm diameter described for *C. chrysanthoides* in *Flora Reipublicae Popularis Sinicae* (Zhang and Ren, 1998). However, the type specimen of *C. chrysanthoides* lacks flowers but has fruits, and the flower diameter of 4–5.5 cm was described based on other specimens (Ye and Xue, 2013). Population BH2 represents an introduced population from the Nanning Camellia Garden gene bank, while population BH1 occurs at the type specimen collection site in Daqingshan, Longzhou. We therefore identified both populations as *C. chrysanthoides*. For each plant, 2–3 fresh young leaves were collected, dried in sealed bags with silica gel, and used for total DNA extraction. Material sources and voucher specimen information are detailed in Table 1 , and population distribution locations are shown in Figure 1 [Figure 1: see original paper]. Voucher specimens are deposited at the Guangxi Institute of Botany Herbarium (IBK).

2.2 DNA Extraction and SSR Genotyping

Total DNA was extracted from leaf tissues using a modified CTAB method (Doyle, 1987). From 59 microsatellite primer pairs developed for *Camellia pingguoensis* and *C. flavida* (Lu et al., 2014; Liufu et al., 2014), we screened 11 pairs that produced clear amplification bands and high polymorphism for this study. PCR amplification protocols followed the procedures described in Liufu et al. (2014).

2.3 Data Analysis

Microsatellite genotyping data were analyzed using Genepop version 4.1 (Rousset, 2008) to test for Hardy-Weinberg equilibrium (HWE) with sequential Bonferroni correction (Rice, 1989). GenALEx 6.5 (Peakall and Smouse, 2012) was

used to calculate diversity indices including mean number of alleles (NA), effective number of alleles (NE), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F), and percentage of polymorphic loci (PPB). Analysis of molecular variance (AMOVA) (Excoffier et al., 1992) and pairwise genetic differentiation coefficients (FST) among species and populations were calculated using Arlequin 3.0 (Excoffier et al., 2005). Gene flow (Nm) was estimated using the formula Nm = (1 - FST) / 4FST.

Principal coordinates analysis (PCoA) was performed using GenALEx 6.5. STRUCTURE 2.3 (Pritchard et al., 2000) was used to analyze the seven populations and infer population genetic structure based on genetic composition differences. Parameters were set as follows: K = 1–6, 20 independent runs for each K, burn-in of 10 iterations, and Markov Chain Monte Carlo (MCMC) of 5 × 10 iterations. Results were analyzed using the online software STRUCTURE HARVESTER (Earl and Vonholdt, 2012) to determine the optimal number of genetic groups (K). Mantel tests in GenALEx 6.5 were used to examine whether genetic distance was correlated with geographic distance (isolation by distance, IBD) among the seven populations.

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3. Results

3.1 Genetic Diversity

A total of 92 alleles were detected at 11 loci across 184 individuals of the three camellia species, with an average of 8.364 alleles per locus (Table 2). Among 77 Hardy-Weinberg equilibrium tests (11 loci × 7 populations), nine showed significant deviation ($P < 0.05$), but only locus TER8 in population NB3 remained significant after sequential Bonferroni correction. All data were used for subsequent analyses.

Population genetic diversity indices are presented in Table 3 . At the species level, *C. parvipetala* showed higher values than *C. chrysanthoides* and *C. micrantha* for mean number of alleles (NA = 3.9), effective number of alleles (NE = 2.328), observed heterozygosity (Ho = 0.520), and expected heterozygosity (He = 0.501). At the population level, mean number of alleles (NA) ranged from 2.7 (NB1) to 5.1 (NB3) with an average of 3.8. Effective number of alleles (NE) ranged from 1.788 (NB1) to 2.466 (PT1) with an average of 2.161. Observed heterozygosity (Ho) ranged from 0.409 (NB1) to 0.543 (NB3) with an average of 0.487. Expected heterozygosity (He) ranged from 0.379 to 0.543 with an average of 0.471, peaking in population NB3 and reaching its minimum in population NB1 (Table 3).

3.2 Genetic Structure

AMOVA results showed that 9.66% of variation occurred among species, 24.62% among populations within species, and 65.72% within populations (Table 4

). Within each species, most molecular variation resided within populations: 75.59% within and 24.41% among populations for *C. chrysanthoides*; 81.80% within and 18.20% among populations for *C. micrantha*; and 64.61% within and 35.39% among populations for *C. parvipetala*.

Pairwise genetic differentiation coefficients (FST) and gene flow (Nm) are shown in Table 5 . FST values ranged from 0.1437 to 0.4533, with one pair showing moderate differentiation (FST $<$ 0.15), six pairs showing large differentiation (0.15 $<$ FST $<$ 0.25), and 14 pairs showing very large differentiation (FST $>$ 0.25), indicating substantial differentiation among populations. Nm values ranged from 0.3015 to 1.4889, with only three pairs showing gene flow greater than 1, indicating low interpopulation gene flow. Interspecific differentiation coefficients were 0.1731 between *C. chrysanthoides* and *C. micrantha*, 0.2583 between *C. chrysanthoides* and *C. parvipetala*, and 0.2068 between *C. micrantha* and *C. parvipetala*, showing smaller differentiation between the former pair and larger differentiation involving *C. parvipetala*.

STRUCTURE and PCoA analyses yielded consistent results regarding genetic structure. STRUCTURE analysis indicated that the optimal number of genetic groups was K = 2 for the 184 individuals from seven populations (Figure 2 [Figure 2: see original paper]). At K = 2, most individuals from the two *C. chrysanthoides* populations and three *C. micrantha* populations clustered together, while most individuals from the two *C. parvipetala* populations formed a separate cluster (Figure 3 [Figure 3: see original paper]).

PCoA results (Figure 4 [Figure 4: see original paper]) divided all individuals into two groups: the two *C. chrysanthoides* populations and three *C. micrantha* populations formed one group, while the two *C. parvipetala* populations formed another. However, the two *C. parvipetala* populations were clearly separated, with population PT1 being more similar to the three *C. micrantha* populations. The first two coordinates explained 18.76% and 12.59% of the total variation, respectively. Mantel test results (Figure 5 [Figure 5: see original paper]) showed a weak positive but non-significant correlation between geographic and genetic distances among the seven populations (R^2 = 0.0847, P = 0.23).

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4. Discussion

4.1 Genetic Diversity

Genetic diversity levels are crucial determinants of a population' s adaptive evolutionary potential (Frankham et al., 2002). Generally, endemic, rare, and endangered plants, as well as small and isolated populations, exhibit low genetic diversity (Spielman et al., 2004; Gao et al., 2017). Compared with congeneric species, the three camellia species showed relatively low mean number of alleles (NA) and expected heterozygosity (He): *C. chrysanthoides* (NA = 3.7, He = 0.431), *C. micrantha* (NA = 3.8, He = 0.476), and *C. parvipetala* (NA = 3.9, He

= 0.501). These values are lower than those reported for *Camellia flavida* (A = 4.4, He = 0.555) (Lu, 2015), *C. taliensis* (AR = 6.776, Hs = 0.597) (Zhao et al., 2014), *C. japonica* (A = 16.5, He = 0.84) (Ueno et al., 2000), and *C. sinensis* (A = 4.3, He = 0.64) (Yao et al., 2012). Thus, all three camellia species exhibited relatively low genetic diversity. Multiple factors influence species genetic diversity, including habitat conditions, geographic distribution, and breeding systems (Nybom, 2004). The three camellia species have extremely narrow distributions, and human transplantation of wild plants combined with land development has reduced wild population sizes and created fragmented distributions, with all populations containing fewer than 100 individuals. Narrow distribution, small population size, and fragmented distribution likely contributed to the low genetic diversity observed in these three camellia species.

4.2 Genetic Structure

High levels of genetic differentiation occurred among populations of the three camellia species (Table 5). Only populations NB1 and NB2 showed moderate differentiation (FST < 0.15), while other population pairs exhibited large (0.15 < FST < 0.25 for six pairs) or very large (FST > 0.25 for 14 pairs) genetic differentiation (Wright, 1968). Within-species differentiation was large for *C. chrysanthoides* (0.2441) and *C. micrantha* (0.1437, 0.2555, and 0.1612), and very large for *C. parvipetala* (0.3514), exceeding most intraspecific differentiation values. This may result from population PT2 being a long-term isolated small population. Such large genetic differentiation has also been detected in the congeneric species *C. flavida* (Lu, 2015). Multiple factors influence population genetic differentiation, with gene flow being one of the most important (Chen, 2000). Gene flow (Nm) among the seven populations was low (Table 5), with only three population pairs showing Nm > 1. According to Wright' s (1931) theory, Nm > 1 is required for gene flow to counteract genetic drift and prevent differentiation. Pollen dispersal and seed dispersal are the two main forms of gene flow in plants. In studies of congeneric species, limited seed or pollen dispersal in *C. flavida* (Wei et al., 2017; Peng and Tang, 2017), *C. oleifera* (Deng et al., 2010), and *C. taliensis* (Liu et al., 2012) resulted in low gene flow and genetic differentiation among populations. The fragmented distribution of the three camellia species restricts pollen and seed dispersal among populations, leading to low gene flow. Their small, isolated wild populations are also subject to strong genetic drift. Fragmented distribution, limited dispersal capacity, small population size, and genetic drift likely contributed to the high levels of interpopulation genetic differentiation observed.

STRUCTURE and PCoA analyses produced similar results, with the optimal number of genetic groups being two. Most individuals of *C. chrysanthoides* and *C. micrantha* formed one group, while most *C. parvipetala* individuals formed another, corresponding to their geographic distribution ranges. This indicates smaller differentiation between *C. chrysanthoides* and *C. micrantha* but larger differentiation between *C. parvipetala* and the other two species. The two sam-

pled populations of *C. chrysanthoides* and three representative populations of *C. micrantha* likely belong to the same species.

4.3 Conservation Implications

Species genetic diversity levels are closely related to their viability and adaptability (Hamrick and Godt, 1996). This study revealed low genetic diversity and high interpopulation genetic differentiation in the three camellia species. Field surveys found that habitats of all sampled populations have been damaged to varying degrees. For example, the distribution area of population PT2 has been converted to star anise plantations; population BH1' s habitat has been converted to eucalyptus plantations; and a highway is planned through population BH2' s distribution area, which would result in removal of most individuals if implemented. Therefore, all extant populations should receive either in situ or ex situ conservation measures as soon as possible based on their actual conditions. When implementing ex situ conservation, representative individuals from each population should be selected for transfer to germplasm resource banks to preserve their genetic resources.

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Note: Figure translations are in progress. See original paper for figures.