Fine-scale spatial genetic structure and gene flow in the karst shade-tolerant shrub *Camellia flavida* (postprint)

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Abstract

Camellia flavida is a golden camellia species with light yellow petals and a typical shade-tolerant shrub in karst habitats. Based on a 15 hm² (300 m × 500 m) monitoring plot in the karst seasonal rainforest of Nonggang, Guangxi, and using 13 microsatellite (SSR) markers, this study investigated the effects of karst landforms on the fine-scale spatial genetic structure (SGS), gene flow, and small-scale genetic differentiation of C. flavida. The results showed: (1) C. flavida exhibited significant SGS within 50 m, with a moderate SGS intensity ($Sp = 0.0248$) among shrub species and seed-dispersal-dependent species; (2) The average dispersal distances of seeds and pollen were relatively short, being 12.47 m and 29.03 m, respectively, with 72.2% of pollen and 81.0% of seeds dispersed mainly within 20 m; (3) Four populations of C. flavida at a small scale (inter-population distance < 1 km), and even two patches 100 m apart, exhibited significant genetic differentiation. Karst landforms hinder gene flow in C. flavida, thereby leading to genetic differentiation at small scales.

Full Text

Preamble

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Fine-Scale Spatial Genetic Structure and Gene Flow of *Camellia flavida*, a Shade-Tolerant Shrub in Karst

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Abstract

Camellia flavida is a golden camellia with yellow petals and a typical shade-tolerant shrub in karst regions. This study aimed to explore whether karst habitat heterogeneity contributes to the fine-scale spatial genetic structure (SGS), gene flow, and genetic differentiation of *C. flavida* at a small scale within a 15 hm² (300 m × 500 m) plot of northern tropical karst seasonal rainforest in Nonggang, Guangxi, based on 13 microsatellites (SSR). The results indicated that there was significant SGS in *C. flavida* within a distance of 50 m, with moderate intensity ($Sp = 0.0248$) among shrub and gravity-dispersed species. The mean seed and pollen dispersal distances of *C. flavida* were short at 12.47 m and 29.03 m, respectively, with 72.2% of pollen and 81.0% of seed being mainly spread within 20 m. In addition, there was significant genetic differentiation among four populations at the small scale (distance between populations < 1 km), and even between two patches separated by a distance of only 100 m. It is concluded that karst habitat heterogeneity significantly influences the gene flow of *C. flavida*, leading to comparatively strong SGS and genetic differentiation at the small scale.

Keywords: karst; *Camellia flavida*; spatial genetic structure; gene flow

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Introduction

China has the world' s largest distribution area of karst landforms, accounting for a significant proportion of global karst area, mainly distributed in Guangxi, Sichuan, and other regions [1]. Typical karst landforms are widely distributed with peak-cluster depressions, featuring complex and diverse microhabitats and variable community structures, with environmental heterogeneity changing dramatically from peak clusters to depressions [2]. Karst regions exhibit high biodiversity, rich rare and endangered flora and fauna, and numerous endemic species, making the regional flora and biodiversity extremely unique [3]. Guangxi Nonggang National Nature Reserve features typical karst landforms, demonstrating this specialization, which is particularly significant in the Gesneriaceae family and golden camellia plants of Theaceae [5]. Habitat heterogeneity can cause morphological variations in leaf shape, bark, or inflorescence of species under different bedrock and soil conditions [6]. In typical peak-cluster valley ecosystems, the xeromorphic anatomical structure of leaves in karst areas is more pronounced than in non-karst areas [7].

Spatial genetic structure (SGS) refers to the distribution pattern of genotypes in two-dimensional space, often analyzed using spatial autocorrelation of kinship coefficients and geographic distances [8-9]. This helps understand interactions among individuals and between individuals and environment, as well as population dynamics, providing important references for in situ and ex situ conservation of endangered plants [10-11]. SGS is considered a key factor determining short-term evolution of populations [12], as it can affect fitness of offspring from nearby matings, leading to inconsistent adaptation to environmental heterogeneity [13-14]. SGS is the result of long-term interaction between plant characteristics and ecological factors, with numerous influencing factors producing different effects, including low-density seed resources, limited pollen and seed flow, overlapping seed shadows, founder effects, diverse life histories, and random self-thinning [15-17]. Extensive pollen and seed dispersal, on the other hand, weakens SGS [13,16,18-21]. The two main factors affecting SGS are seed and pollen flow [22-24].

Studies on gene flow of karst plants are limited, and even fewer on understory shrubs. *Camellia flavida* is a golden camellia with yellow petals, belonging to typical evergreen shade-tolerant shrubs in karst. It has a narrow distribution and fragmented habitat, typically growing in shaded valley understory or thickets at altitudes of 100-500 m [25]. Pollination is mainly insect-mediated, and seeds are capsules primarily dispersed by gravity. This study takes the shade-tolerant shrub *C. flavida* in karst regions as the research object, based on a 15 hm² (300 m × 500 m) plot in Nonggang northern tropical karst seasonal rainforest, using microsatellites (SSR) to address: (1) What is the pattern of gene flow in *C. flavida*? (2) How does strong habitat heterogeneity of karst affect the SGS and genetic differentiation of *C. flavida*? (3) What are the impacts on gene flow and genetic differentiation?

1. Study Site

The 15 hm² (300 m × 500 m) northern tropical karst seasonal rainforest plot in Guangxi Nonggang was established by the Guangxi Institute of Botany and is part of the Chinese Forest Biodiversity Monitoring Network (CForBio) and the global Center for Tropical Forest Science (CTFS) network, being the only permanent plot monitoring tropical karst forests [26]. It represents typical habitat types of southwestern Guangxi karst regions [2,26], featuring seasonal flooding and a series of slopes from hilltops to depressions. The hilltops have high rock exposure, while the plot includes a depression with thicker soil layers and dense lianas. Coupled with shading effects from vegetation on surrounding slopes, the microhabitat differs significantly from the surrounding slopes [2,27].

The plot was established according to CTFS technical specifications, measuring and recording DBH, names of woody species, growth status, and coordinates [28]. Several *C. flavida* populations are aggregated around the depression within the plot, with additional discontinuously distributed populations around the plot due to habitat heterogeneity. This plot is representative for studying the effects of karst habitat heterogeneity on fine-scale spatial genetic structure and gene flow of *C. flavida*.

2. Survey and Sampling Methods

All *C. flavida* individuals in the 15 hm^2 plot were surveyed and sampled. Leaves were collected and dried in sealed bags with color-changing silica gel, then brought back to the laboratory for DNA extraction. Coordinates of individuals with DBH 1 cm were precisely measured according to CTFS permanent plot construction technical specifications, provided by the Li Xianjun research group from Guangxi Institute of Botany. Coordinates of individuals with DBH < 2 cm were obtained through geometric calculations by measuring distances to nearby plants with known coordinates.

Based on flowering age, *C. flavida* individuals were divided into three age stages: (1) not reaching flowering age (DBH < 1 cm), (2) partially reaching flowering age (1 cm < DBH < 2.5 cm), and (3) all reaching flowering age (DBH 2.5 cm).

3. Genetic Analysis

DNA extraction used a modified PCR method [29]. Based on published primers for *C. flavida* [30] and related species *Camellia pingguoensis* [31], 13 microsatellite loci were selected. A total of 38 *C. flavida* individuals were used as test samples for PCR amplification and denaturing polyacrylamide gel electrophoresis genotyping. Loci with clear amplification bands and high polymorphism were selected for analysis of all samples.

4. Data Analysis

4.1 Genetic Diversity

The online software GenePop on the Web [32] was used to test for null alleles and Hardy-Weinberg equilibrium (HWE). Micro-Checker v2.2.3 software [34] was used to detect genotyping errors. GenAlEx 6 software [33] was used to calculate the number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_IS).

4.2 Spatial Genetic Structure

SPAGeDi software [35] was used to calculate Loiselle et al.'s [9] kinship coefficient (F_ij) to analyze the spatial genetic structure of *C. flavida*. Parameters were set as follows: distance class = 10 m, 1000 simulation statistics. The Sp statistic was used to measure SGS intensity, calculated as Sp = -b_F/(1 - F_1), where b_F is the slope of F_ij against the natural logarithm of distance, and F_1 is the kinship coefficient of the first distance class [36].

4.3 Small-Scale Genetic Differentiation

Four *C. flavida* populations in the Nonggang Nature Reserve were studied: NG (144 individuals), CS (219 individuals), PA (35 individuals), and PB (62 individuals). Inter-population distances ranged from 0.296 to 0.982 km (mean 0.627

km, all < 1 km). GenAlEx 6.5 software [33] was used to calculate genetic differentiation coefficient (F_ST) and gene flow (Nm). STRUCTURE v2.2 software [37] was used to simulate population genetic structure. Parameters were set as: Burn-in = 100,000, Markov' s chain Monte Carlo (MCMC) = 500,000, K = 1-5. Results were compressed and analyzed using the online software Structure Harvester [38] to determine the optimal number of genetic populations.

4.4 Seed and Pollen Flow

Seedlings in the 15 hm^2 plot were considered offspring, while saplings and adult trees were considered candidate parents. CERVUS 3.07 software [40] was used for parentage analysis. CERVUS uses maximum likelihood methods, estimating the logarithm of likelihood values (LOD) between offspring and parents, with significance determined through simulation. In parentage analysis with unknown parental sex, the parent pair with the highest LOD value is considered the true parent pair. Parentage analysis simulation parameters were set as: offspring number = 21, candidate parents = 48, locus mismatch rate = 0.01, confidence level = 0.80. Based on Ashley' s [42] method and the gravity-dependent seed dispersal characteristics of *C. flavida*, two assumptions were made: (1) both parents found - the closer is the maternal parent, the farther is the paternal parent; (2) only one parent found - considered as the maternal parent. Pollen and seed flow patterns were then mapped using coordinates of offspring, maternal, and paternal parents. GenAlEx 6.05 software [34] was used to calculate pollen and seed dispersal distances.

5. Results

5.1 Spatial Distribution

A total of 460 *C. flavida* individuals were distributed in the 15 hm^2 plot, with seedlings, saplings, and adult trees numbering 21, 48, and 391, respectively. Individuals were aggregated around the depression (100 m × 300 m) and were clearly divided into two patches by the species-dense, seasonally flooded depression. No *C. flavida* was distributed outside the plot. NG2 was a relatively isolated population.

5.2 Genetic Diversity

Using 13 loci with clear amplification bands and high polymorphism, 460 individuals from the 15 hm^2 plot were amplified. The mean number of alleles, observed heterozygosity, expected heterozygosity, and fixation coefficient were 7.23, 0.748, 0.729, and -0.027, respectively. GenePop on the Web detected no null alleles at any loci. After Bonferroni correction, only primer FLA26 significantly deviated from Hardy-Weinberg equilibrium ($P < 0.05$). Primers showed high polymorphism and reliability (Table 1).

Table 1 Genetic diversity of *C. flavida* in the 15 hm^2 plot

Primer	Primer Sequence (5 -3)	Ta (°C)	HWE (P value)	Alleles	H_O	H_E	F_IS
... (table data preserved as in original) ...							

5.3 Spatial Genetic Structure

The kinship coefficient (F__ij) between *C. flavida* individuals showed significant positive correlation within 10-50 m (F__ij = 0.0625), slightly lower than first cousins. From 50-130 m, F__ij was in significant or non-significant negative correlation. From 130-220 m, F__ij showed significant negative correlation. The Sp statistic for SGS intensity was 0.0248.

5.4 Small-Scale Genetic Differentiation

F-statistics showed significant genetic differentiation among the four *C. flavida* populations at small scale (F__ST = 0.116, $P < 0.05$). STRUCTURE clustering analysis indicated $K = 2$ as the optimal number of genetic groups, showing genetic differentiation between the two patches (F__ST = 0.079, $P < 0.05$) [FIGURE 4].

Table 2 Genetic differentiation coefficient (F__ST) and gene flow (Nm) among *C. flavida* populations at small scale

Population	NG	CS	PA	PB
NG	-	1.679	2.120	1.632
CS	0.130*	-	2.285	1.754
PA	0.133*	0.105*	-	2.161
PB	0.099*	0.125*	0.104*	-

Note: Lower diagonal shows F__ST; upper diagonal shows Nm; "*" indicates significant genetic differentiation ($P < 0.05$).

5.5 Seed and Pollen Flow

Parentage analysis of seedlings in the 15 hm^2 plot showed that at trio confidence level, 85.7% of seedlings had pollen sources within the plot, while 14.3% may have come from outside the plot. Only one parent was found for most seedlings. Full-sibs and half-sibs accounted for only 33.3% of all seedlings.

Pollen and seed dispersal distances were calculated from coordinates of offspring, maternal, and paternal parents. Seed dispersal distance ranged 0.65-51.05 m (mean 12.47 m), while pollen dispersal distance ranged 3.06-194.73 m (mean 29.03 m). Both pollen and seed dispersal were mainly within 20 m, accounting for 72.2% and 81.0%, respectively [FIGURE 5, TABLE 4].

Table 3 Parentage analysis of seedlings in the 15 hm² plot

Offspring	First Candidate	Pair Loci Mismatching	Pair LOD Score	Pair Confidence	Second Candidate	Trio Loci Mismatching	Trio LOD Score	Trio Confidence
... (table data preserved as in original) ...								

Table 4 Seed and pollen dispersal distances of *C. flavida*

Offspring	Mother	Father	Seed Dispersal Distance (m)	Pollen Dispersal Distance (m)
···(table data preserved as in original) ···				

Analysis of paternal sources for seedlings from the two patches showed that most pollen flow occurred within patches rather than between patches [FIGURE 6].

6. Discussion

6.1 Spatial Genetic Structure

The two main factors affecting SGS are seed flow and pollen flow [22-24]. Strong pollen flow often produces weak or no SGS, as seen in wind-pollinated plants like chestnut and chinquapin [43] and Brazilian pine [45]. When pollen and seed are highly localized, strong SGS is produced, as in insect-pollinated plants like wild soybean [46] and blackwood [11]. *Camellia flavida* relies mainly on insect pollination and gravity-dependent seed dispersal with habitat preference, thus predicted to have strong SGS.

Results showed significant SGS within 50 m (Sp = 0.0248), with short pollen and seed dispersal distances (12.47 m and 29.03 m, respectively), and 72.2% of pollen and 81.0% of seed dispersed within 20 m. Limited pollen and seed dispersal led to strong SGS. The Sp value of *C. flavida* (0.0248) is similar to that of *Handeliodendron bodinieri* (0.0233) [49] and within the range summarized by Vekemans and Hardy [36] for gravity-dispersed shrubs (0.0281), indicating moderate SGS among shrub and seed-dependent species.

6.2 Small-Scale Population Genetic Differentiation

Typical karst landforms have strong heterogeneity in space, soil, and water. Plants show obvious adaptation to the special and rich microhabitats of karst [50]. Studying genetic differentiation at small scales in karst regions helps understand species adaptation and the richness of karst endemics. This study found significant genetic differentiation between two patches separated by only 100 m in the 15 hm² plot (F_ST = 0.079, P < 0.05), and moderate but significant differentiation among four populations at small scale (<1 km) (F_ST = 0.116, P < 0.05).

Linhart and Grant [52] summarized that gene flow and natural selection strongly influence genetic differentiation at 100-300 m scales in woody plants. Gao et al. [53] found similar genetic differentiation in the *Primulina eburnea* complex in southern China karst due to genetic drift and limited gene flow. *Camellia flavida* follows this pattern: strong karst habitat heterogeneity and habitat preference cause fragmented distribution, reducing gene exchange. The depression between the two patches in the 15 hm² plot has different microhabitat conditions (seasonal waterlogging, high population density) [27], which hinders seed germination and seedling growth while effectively blocking pollen dispersal between patches. Different slope aspects and vegetation types create habitat heterogeneity and different selective pressures, generating genetic differentiation.

6.3 Pollen and Seed Flow

Seed and pollen flow are fundamental ecological processes determining genetic diversity, evolution, and adaptation. Pollen dispersal patterns in karst regions and the impact of peak-cluster landforms are important research questions. Increasing evidence shows pollen dispersal distances are greater than previously thought [54-55], with many tropical trees dispersing pollen hundreds of meters to kilometers [54]. However, *C. flavida* in karst regions showed short seed and pollen dispersal distances (12.47 m and 29.03 m), indicating limited gene flow. This suggests that karst landforms hinder gene flow in *C. flavida*, possibly due to strong habitat heterogeneity, shrub characteristics, and habitat preference. While canopy trees may have less obstruction for pollen dispersal, understory shrubs like *C. flavida* grow in species-rich, heterogeneous habitats where pollinators cannot easily find distant flowers, limiting pollination to very small ranges.

7. Conclusion

Few studies have examined whether typical karst habitat heterogeneity affects gene flow in shade-tolerant shrubs. This study found that *C. flavida* has short pollen and seed dispersal distances, leading to significant genetic differentiation at small scales (100 m). This demonstrates that karst habitat heterogeneity significantly influences gene flow in *C. flavida*, and the shrub' s characteristics and habitat preference are closely related to gene flow patterns.

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References

[1] ···(references preserved as in original) ···

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

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