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Genetic diversity and differentiation of *Quercus semiserrata* Roxb. in northern Thailand revealed by nuclear and chloroplast microsatellite markers

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Abstract

This study describes the level of genetic diversity and differentiation of 10 populations of *Quercus semiserrata* Roxb. in northern Thailand, divided into four groups (A–D) based on their geographical location. Variation at eight nuclear (nSSR) and nine chloroplast (cpSSR) microsatellite loci was examined. The eight nuclear microsatellite loci employed detected a total of 139 alleles ($n = 392$), and 16 haplotypes based on length variants at the nine cpSSR loci were identified. Populations in group B harbored the highest gene diversity (H) and allelic richness (Ar) in both their nSSRs and cpSSRs (nSSR, $H = 0.79$ and Ar = 7.3; cpSSR, $H = 0.5$ and Ar = 2.5), the nSSRs of populations in group C had the lowest gene diversity and allelic richness, while the cpSSRs of populations in group A had the lowest gene diversity and haplotypic richness. Calculations (AMOVA) of each population's contributions to the total diversity and allelic richness indicated that most (86.62%) of the variation in nSSRs was due to differences within populations, but most (63.72%) of the total genetic variation in chloroplast haplotypes was due to differences among populations within groups. The ratio of pollen flow to seed flow was estimated to be 94:1. Pairwise $F_{ST}/(1 - F_{ST})$ values were significantly correlated with geographic distances among populations for cpSSRs ($r = 0.337$; $P < 0.05$), but not nSSRs. UPGMA and NJ phylogenetic trees based on Nei's genetic distances for nSSRs were constructed. The results suggest that four populations in group B (Khun Wang, Obluang, Doi Suthep and Doi Inthanon) had the highest genetic diversity and high numbers of chloroplast haplotypes. Therefore, these populations should be given the highest priority for conservation of this species. This is the first report concerning the genetic diversity and differentiation of this species, and provides basic genetic information that should facilitate attempts to conserve the species.

Keywords: cpDNA; Genetic diversity; Microsatellites; Rare species; Tropical tree

1. Introduction

There are increasing global concerns about deforestation, and associated losses of species diversity in tropical forest ecosystems (Rajora, 1999), caused by population pressures, shifting cultivation practices, agricultural development, forest colonization, forest fires and unsustainable, inadequately supervised logging practices. Between 1980 and 1990 tropical forests were destroyed at a global average rate of more than 0.8% per annum, implying that the area of tropical forests has diminished by a 10th during the last 12 years (FAO, 1997). Deforestation can eliminate entire populations of some species and reduce the genetic diversity of residual populations (Kanowski, 1999), which is potentially catastrophic since genetic diversity is the basis of all biodiversity and is widely recognized as a key requirement for the long-term survival of species on an evolutionary time-scale. It provides the template for adaptation, evolution and survival of populations and species, especially in environments that are subject to climate changes or the introduction of new pests, pathogens or competitors (Rajora and Mosseler, 2001). Thus, conserving genetic diversity is one of the most profound challenges facing forest managers relying on either natural or artificial regeneration systems. Tree improvement programs, and disturbances such as those listed above, can significantly affect genetic variability in subsequent forest populations (Rajora, 1999). Therefore, it is extremely important to understand the processes that adversely affect population and

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genetic variation and to take effective counter-measures where possible.

Genetic analysis of populations requires suitable multivariate markers that can elucidate fine-scale details of spatial structure (Streiff et al., 1998) and reconstruct gene flow patterns (Streiff et al., 1999). For plants, comparative analyses of nuclear and chloroplast microsatellites have become popular approaches for such purposes because they can provide complementary and often contrasting information on the genetic structure, differentiation and gene flow (pollen- and seed-mediated) within and among their populations (Birky, 1988; McCauley, 1995; Ennos et al., 1999; Weising and Gardner, 1999; Ishii et al., 2001; Lira et al., 2003; Ueno et al., 2005). Genetic diversity and differentiation measures derived from these kinds of markers may greatly facilitate decisions regarding conservation priorities for populations of threatened species (Petit et al., 1998).

The nuclear genome is biparentally inherited and reflects both seed and pollen gene flow (Ouborg et al., 1999; Petit et al., 2005). Variation at neutral loci (point and/or indel mutations) within the nuclear genome offers great potential for gene flow studies. Nuclear-encoded variants are codominant and in many cases can be phylogenetically interpreted (Goldstein et al., 1995; Olsen and Schaal, 2001). These properties make nuclear loci suitable for estimating a range of indirect gene flow parameters using differentiation, private allele or phylogenetic models. Furthemore, if loci harbor high levels of polymorphism they can be used to calculate direct estimates of gene flow.

In angiosperms, chloroplast genomes are predominantly maternally inherited (mainly transmitted through seeds) and thus can reveal maternal lineages (Birky, 1995; Dumolin et al., 1995; Radetzky, 1990; Rajora and Dancik, 1992). In addition, they are particularly sensitive to the effects of fragmentation; partly because they have smaller effective population sizes than nuclear genomes and partly because seed-mediated gene dispersal is usually more limited than pollen-mediated gene flow. Consequently, chloroplast-specific markers should theoretically provide good indicators of historical bottlenecks, founder effects and genetic drift (Li et al., 2007). However, a major drawback of using chloroplast-specific markers is that chloroplast genomes tend to have low mutation rates, and until relatively recently this has severely limited the amounts of cytoplasmic variation detected at sub-species levels (Wolfe et al., 1987). However, the discovery of polymorphic mononucleotide repeats in plant chloroplast genomes, which display length variation in the number of repeats, analogous to mutation models exhibited by nuclear microsatellites, has offered new opportunities to study cytoplasmic variation at high resolution (Provan et al., 1999, 2001; Lira et al., 2003).

The aims of this study were to assess the genetic diversity and differentiation of *Quercus semiserrata* Roxb. populations in northern Thailand using chloroplast and nuclear microsatellites, and to suggest guidelines for conserving the species using the acquired genetic information.

2. Materials and methods

2.1. Study species

Q. semiserrata Roxb. (Fagaceae) is being planted in northern Thailand as a ''framework species'' (one of 20–30 native tree species being planted in mixtures to provide a framework for re-establishing biodiversity) following nursery and field trials at the Forest Restoration Research Unit of Chiang Mai University, Thailand (Elliott et al., 2003). It is a large, late successional, evergreen tree with a dense crown and straight stem, reaching heights up to 30 m and diameters at breast height of up to 100 cm. It occurs in scattered locations in mixed evergreen/deciduous and deciduous evergreen/pine forests, at elevations of 850–1400 m, in northern Thailand, and in various other wet, tropical hill forests in India, Myanmar and Indo-China (Forest Restoration Research Unit, 2000). Its ovaries have 4–6 styles, up to 3.5 mm long, with villous-tomentose, brown-shaped or hemispherical cupules, 2–3 cm in diameter and up to 2.5 cm high, with 6–8 broad rings. Acorns are ovate-cylindrical, 4 cm long and 2 cm in diameter (Menitsky, 2005). Flowering occurs in spring, in the hot, dry season before the onset of monsoon rains, fruiting from November to March. As a rule, the acorns drop in January simultaneously with partial leaf shedding. The wood is very heavy and hard; sapwood is reddish-grey and heartwood reddish to reddish-brown; rays very narrow. The wood is used for constructing houses and vehicles for transporting goods.

2.2. Sample collection and DNA extraction

Young leaves were collected in April 2005 from *Q. semiserrata* trees in populations at the following 10 locations in northern Thailand (Fig. 1): Nam Nao National Park (1) (16°42′49.23″N, 101°33′ 57.16″E), Khun Wang Royal Agricultural Research Center (2) (18°37′52.02″N, 98°29′49.68″E), Obluang National Park (3) (18°20′36.01″N, 98°29′28.88″E), Doi Suthep-Pui National Park (4) (18°49′31.34″N, 98°53′41.16″E), Doi Inthanon National Park (5) (18°30′28.97″N, 98°31′25.06″E), Chae Sawn National Park (6) (18°47′45.60″N, 99°30′47.52″E), Khun Chae National Park (7) (19°25′29.04″N, 99°27′11.52″E), Doi Chiang Dao Wildlife Sanctuary (8) (18°26′5.18″N, 98°53′45.63″E), Pang Ma O (9) (19°22′11.13″N, 98°42′29.32″E) and Doi Wieng Pha National Park (10) (19°44′45.68″N, 99°11′10.46″E). The number of trees sampled in each population varied from 11 in Doi Wieng Pha National Park (10) to 52 in Chae Sawn National Park (6). Total genomic DNA was extracted from the leaves of each sampled tree using the modified CTAB method described by Murray and Thompson (1980).

2.3. Microsatellite markers and genotyping

Eight nuclear microsatellite markers were selected for genotyping *Q. semiserrata*: Qm50-3M developed for *Quercus myrsinifolia* (Isagi and Suhandono, 1997), CA15 developed for