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# Gene flow pattern and mating system in a small population of *Quercus semiserrata* Roxb. (Fagaceae)

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A B S T R A C T

Pollen flow from external sources is important for the conservation of tree species in fragmented forests or small populations, because it can be sufficient to prevent differentiation among them, and appears to be able to prevent the loss of their genetic diversity through genetic drift. In this study, we examined the genetic heterogeneity of pollen pools accepted by each *Quercus semiserrata* seed parent at the Khun Wang Royal Agriculture Research Center, Thailand, both within and among two mast fruiting years (2005 and 2007), using paternity analysis and analysis of molecular variance (AMOVA). The mating systems of the trees were also examined using the multilocus mating system model (MLTR), after determining the genotypes at eight microsatellite loci of 26 seed-trees and 435 seeds from 8 seed-trees in the 2 mast fruiting years. The average distance of effective pollen flow within the plot was estimated to be 52.4 m, and 95% of effective pollen was dispersed within 200 m, indicating that effective pollen flow is highly localized and that most effective pollen is contributed by near-neighbor trees. The proportion of effective pollen that immigrated from external sources was estimated to be 26.2%. The AMOVA analysis based on the pollen haplotypes showed that the pollen pools, both total and for each reproductive year, significantly genetically differed among the seed parents. Using a mixed mating model, the estimate of biparental inbreeding for the total population ($t_m - t_s$) was 0.013, indicating that a low proportion of mating occurred among close relatives. The effective number of pollen donors ($N_{ep}$) was estimated to be 9.987 using the TwoGener model, or 10.989 using the mixed mating model. The effective number of pollen donors of seeds was higher in the mast fruiting year 2005 than in the other examined year, 2007. Consequently the allelic richness and genetic diversity of seeds produced in 2005 were higher than those produced in 2007. Overall, the results show that high outcrossing rates, high levels of gene flow from other populations and heterogeneity in the pollen received by an individual may enhance the ability of populations to maintain effective population sizes. Therefore, these processes may be sufficient to prevent loss of genetic diversity through genetic drift of *Q. semiserrata* at this study site.

## 1. Introduction

Gene movement among populations (i.e. gene flow) and within populations can significantly affect evolutionary processes such as natural selection and random genetic drift. Consequently it can have important effects on the spatial genetic structure of populations. However, the magnitude of effects of drift and selection on patterns of genetic variation will depend on the reproductive ability of the organism and the rate of gene flow (Gaiotto et al., 2003). In plant species that produce large, immobile seeds (e.g. oaks, hickory and walnut), pollen flow is probably the most important component of gene flow (Dow and Ashley, 1998). Pollen flow from external sources is important for the conservation of tree species in fragmented forests or small populations, because it can be sufficient to prevent differentiation among them, and appears to be able to prevent the loss of their genetic diversity through genetic drift (Hamrick et al., 1989).

Microsatellites have become the preferred markers for studying the genetic diversity of natural plant populations, quantifying gene flow among them, and identifying populations that should be prioritized for conservation (Dow et al., 1995; Chase et al., 1996; Dayanandan et al., 1997; Streiff et al., 1998; Ueno et al., 2000). Furthermore, microsatellite loci tend to harbor high levels of polymorphism, and thus are useful for identifying pollen parents and characterizing pollen flow at local scales. Several methods have been developed for directly measuring pollen-mediated gene flow and distances of pollen dispersal, most of which rely on paternity exclusion (Smith and Adams, 1983; Devlin and Ellstrand,

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1990; Burczyk and Chybicki, 2004; Marshall et al., 1998) or paternity assignment (Devlin et al., 1988; Chase et al., 1996; Dow and Ashley, 1998; Streiff et al., 1999). CERVUS 2.0 (Marshall et al., 1998) is widely used software for paternity analysis based on the former approach, i.e. determining the parents of offspring by comparing genotypes of candidate fathers and offspring after subtracting the maternal contribution to each offspring.

In wind-pollinated woody plant species, pollen flow often occurs over long distance and generally depends on the distance and direction of pollen parents relative to the seed parent (Streiff et al., 1999; Burczyk and Chybicki, 2004). In addition, since there is often substantial interannual variation in flowering at individual and pollen levels, the genetic composition of the pollen pools accepted by different seed parents is likely to differ both within and among reproductive years (Streiff et al., 1999). Therefore, single-season studies may provide limited indications of mating patterns. The effective pollen donor size per generation is of interest, since annual variations in pollen composition received by an individual are likely to increase genetic variation among progeny (Irwin et al., 2003). Recently, Smouse et al. (2001) developed a new model of molecular variance, dubbed the TwoGener model (parent–offspring), designed to facilitate the characterization of pollination patterns using relatively small samples of offspring. This model provides an indirect method of estimating pollen movement that is a hybrid of traditional paternity analysis and genetic structure analysis, allowing us to quantify heterogeneity among the pollen gene pools of seed samples from individual seed parents scattered across the landscape, in association with dispersion functions. This method does not require knowledge of the genotypes of all seed parent trees in the studied plot. In addition, the TwoGener model allows effective numbers of pollen donors and mean pollination distances to be estimated.

The mating system is a key determinant of the spatial genetic structure within and among populations, and strongly influences both the extent of inbreeding and genetic differentiation among populations. Knowledge of mating systems is helpful for forest conservation, tree breeding, and targeted seed collection for environmental reforestation strategies (Bittencourt and Sebbenn, 2007). The mixed mating system model, as implemented in MLTR software (Ritland, 2002, 2004) is widely used to examine the mating systems of plant species. In this model, the mating system of plants can be summarized by estimates of the outcrossing rate, mating with relatives, and the probability of two progeny having the same mother and father (Ritland and Jain, 1981). In addition, information about the probability that two progenies share the same father can reveal the extent of diversity in pollen donor pools, and provide indications of the effective number of pollen donors for a given maternal plant (Ritland, 1989). Simultaneous use of paternity analysis, the TwoGener model (AMOVA) and the mixed mating system model can provide complementary information on the temporal and spatial genetic heterogeneity of pollen flow, variations in pollen pools at local scales and pollen dispersal distances.

*Quercus semiserrata* Roxb. (Fagaceae) 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 various other wet, tropical hill forests in India, Myanmar and Indo-China (FORRU, 2000). It 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). Flowering occurs in spring, in the hot, dry season before the onset of monsoon rains, and fruiting from November to March. As a rule, the acorns drop in January simultaneously with partial leaf shedding. We previously examined the genetic diversity and differentiation of *Q. semiserrata* within and between ten populations in northern Thailand using nuclear and chloroplast microsatellite markers (Pakkad et al., 2008). The results suggest that four populations (located at the Khun Wang Royal Agricultural Research Center, Obluang National Park, Doi Suthep National Park and Doi Inthanon National Park) had the highest genetic diversity and high numbers of chloroplast haplotypes, and thus should be given the highest priority for conservation of this species. However, in the previous study we did not examine the species' pollen dispersal and pollen pool patterns, or mating system, although knowledge of these features is essential for understanding the reproductive processes and developing efficient strategies to conserve viable populations. Therefore, the objectives of this study were to investigate the genetic heterogeneity of pollen pools accepted by individual seed parents both within and among reproductive years, and to evaluate the mating system of *Q. semiserrata*, using paternity analysis, analysis of molecular variance (AMOVA) and multilocus mating system analysis (MLTR).

## 2. Materials and methods

### 2.1. Study site

The study site was located in the grounds of the Khun Wang Royal Agriculture Research Center (approximately 18°37′52.02″N, 98°29′49.68″E, Chiang Mai province, Thailand), a primary objective of which is develop alternatives to replace opium poppy cultivation and improve conditions for people living in villages in the surrounding hills. It is situated at 1280 m above sea level along a ridge top and is the highest establishment in the Mae Wang sub-watershed of the Mae Khan watershed. The study site was cleared of evergreen forest that had previously covered it approximately 50 years ago, to provide land for cultivating cabbages, corn, potatoes and other cash crops. There are now sparsely scattered tree across the site, and it is surrounded by a remnant forest. The average temperature and annual rainfall are 19.5 °C and 2137 mm, respectively.

All 26 mature trees (density, *ca.* 2.4 trees/ha) at the study site were mapped, and young leaves were sampled from all of them for DNA extraction. In addition, acorns were collected from eight seed parents in early April in both 2005 and 2007 (mast fruiting years). One hundred acorns per seed parent per year were collected and sown in modular germination trays. The first true leaves produced by all germinated seeds from each seed parent were harvested, stored at −80 °C and used at a later date for DNA extraction. Total genomic DNA was extracted from the leaves of each sampled tree using the modified CTAB method described by Murray and Thompson (1980).

### 2.2. 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 *Q. salicina* (Kawahara, personal communication), ssrQpZag9 and ssrQpZag46 developed for *Q. petraea* (Steinkellner et al., 1997), quru-GA-1C08 and quru-GA-0C19 developed for *Q. rubra* (Preston et al., 2002), and bcqm07 and bcqm96 developed for *Q. magnolica* var. *crispula* (Mishima et al., 2006). The sequences of forward and reverse primers for the locus CA15 were CGGTAAGACGTTTGGTGTAG and TTGTACGGACGCCATTGAAA, respectively.

PCR amplification was performed in 10 μl reaction mixtures containing 10 ng of template DNA, 1× PCR buffer (20 mM Tris–HCl