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Gene flow interruption in a recently human-modified landscape: The value of isolated trees for the maintenance of genetic diversity in a Mexican endemic red oak

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ABSTRACT

Gene flow within and among populations is an important factor to maintain genetic cohesiveness and diversity across landscapes. Nowadays, human land use has led to a large forest conversion, creating many fragmented areas where remnant trees play an important role in conserving biodiversity. In this study, we analyzed the effects of a recent anthropogenic forest fragmentation on the genetic diversity and genetic heterogeneity of pollen pools accepted by individuals of the red oak *Quercus castanea* growing in forest patches and as isolated trees in central Mexico. Pollen movement was also evaluated by the analysis of outcrossing rates using seven nuclear microsatellites. We assumed that adult trees are remnants of the populations that existed previous to the forest fragmentation, while progenies of these trees are the result of recent reproductive events occurring after the fragmentation. We found high genetic diversity in both adult trees and progenies, even though progenies of isolated trees showed a significant reduction in heterozygosity as compared to their mother trees. However, the results of TWOGENER and mating system analyses indicated similar numbers of pollen donors in the progenies of mother trees from fragments and in isolated trees. Overall, our results suggest that gene flow is still extensive among forest fragments and isolated trees, conferring them a great value for the conservation of genetic diversity and connectivity.

1. Introduction

Gene movement within and among populations can have an important influence on the patterns of genetic differentiation and on the evolution of local adaptations in plant species (Streiff et al., 1999; Rieseberg and Burke, 2001; Lenormand, 2002; Sork, 2016). Pollen movement is the most important vector of gene flow in plant species that produce large and relatively immobile seeds (Dow and Ashley, 1998; Broadhurst, 2015), and wind pollinated species usually have the greatest potential for pollen movement over long distances (Ennos, 1985). In particular, wind pollinated trees typically show low levels of genetic differentiation among populations, high genetic variation within populations, and high outcrossing rates, which are features associated with high levels of gene flow (Hamrick and Godt, 1996; Dow and Ashley, 1998). Pollen flow is, therefore, a very important factor in the maintenance of genetic diversity within populations and connectivity among populations in forest trees (Burczyk and Chybicki, 2004; Hamrick, 2004).

However, studies on contemporary pollen movement in forest trees have shown that pollen densities decline rapidly from the source and that the majority of the fertilizations of maternal trees are effected by nearby pollen donors (Smouse and Sork, 2004; Deacon and Cavender-Bares, 2015), even though some proportion of long distance dispersal also occurs (Smouse et al., 2001; Hampe et al., 2013). If a large proportion of pollen movement occurs at a local scale, populations could be genetically subdivided creating local genetic structure (Smouse et al., 2001; Smouse and Sork, 2004; Sork, 2016). In oaks, evidence suggested that most gene flow occurs within moderate distances (Vranckx et al., 2014; Deacon and Cavender-Bares, 2015; Moracho et al., 2016; but see Buschbom et al., 2011) with mating taking place between neighbor

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trees, including relatives (Smouse et al., 2001; Sork et al., 2002; Valbuena-Carabaña et al., 2005).

Human land use for agricultural and urban development has led to large transformation of natural ecosystems (Ottewell et al., 2009). Globally, considerable areas of forests have been transformed into agricultural and grassland areas containing a few scattered remnant trees of the original pre-clearance plant communities (Gibbons and Boak, 2002; Manning et al., 2006). This process leads to an increase in geographic isolation and a reduction in connectivity among forest patches, therefore influencing the amount of gene flow between remnant populations (Herrera-Arroyo et al., 2013). The increase in genetic isolation and the decline in size may threaten the viability of these populations (Dutech et al., 2005). Therefore, changes associated with habitat degradation could result in an erosion of genetic diversity and in an increase in population genetic divergence (Young et al., 1996). Consequently, understanding gene flow at local scales and how landscape changes alter gene movement are very important issues for conservation biology (Fahrig, 1997; Sork et al., 1998, 1999; Smouse et al., 2001; Martins et al., 2016).

If connectivity among forest patches is lost, some other processes such as re-colonization, immigration and inbreeding may also be affected (Henderson et al., 1985; Dunning et al., 1992; Richards, 2000; Wilson et al., 2016). Some of the negative consequences of habitat fragmentation and degradation are genetic bottlenecks and the disruption of breeding systems of plants at both population and individual levels (Jump and Peñuelas, 2006). Remnant long-lived trees are usually surrounded by an unfavorable matrix of modified lands, are isolated from other trees, and contribute little to forest regeneration due to reduced pollination and the disruption of natural seed dispersal (Gibbons et al., 2008). Nevertheless, several studies have recorded pollen flow into pasture trees separated by hundreds of meters, indicating that spatially isolated trees may not necessarily be reproductively isolated (Cascante et al., 2002; Fuchs et al., 2003; Martins et al., 2016). However, if recruitment is virtually absent, these isolated individuals will disappear rapidly in agricultural landscapes and may be lost in 90–180 years (Gibbons et al., 2008; Fischer et al., 2010).

Currently, one of the most common features in Mexican forest landscapes is forest fragmentation. Temperate forests, particularly oak forests, confront serious problems of deforestation and fragmentation (Torres-Miranda et al., 2011). These processes are exemplified in the catchment basin of Lake Cuitzeo located in Michoacán state in central Mexico, where an intense land-use change over the last decades has occurred (Aguilar-Romero et al., 2016). Large continuous oak forests have been reduced to a large number of small patches of variable size due to strong human pressures like urban growth, expansion of the agricultural frontier, and removal of trees for charcoal production (Aguilar-Romero et al., 2012).

In this study, we were interested in knowing how recent forest fragmentation has altered gene movement in *Quercus castanea*, a long-lived oak species occurring in forest fragments of different sizes as well as isolated individuals in agricultural and pasture lands within the Cuitzeo basin (Fig. 1). We chose *Quercus castanea* as a model system due to its abundance and fragmented spatial distribution in the study area. We analyzed adult trees under the assumption that these represent the surviving genotypes that were established before the large-scale fragmentation process, and their respective progenies, which have resulted from recent reproductive events occurring after the fragmentation (Herrera-Arroyo et al., 2013). The aims of this study were (i) to assess the genetic diversity and structure of adults and progeny of *Q. castanea* in four fragments with different sizes (from less than 0.5 ha to more than 100 ha) and of isolated trees, (ii) to estimate the genetic heterogeneity of pollen pools fertilizing mother trees in the different conditions (trees in isolation, and in fragments of different sizes), (iii) to estimate the outcrossing rates of trees under the different fragmentation conditions, and (iv) to generate connectivity maps, based on habitat suitability grids, for trees in forest fragments, isolated trees and both forest fragments and isolated trees.

2. Materials and methods

2.1. Study system and collecting methods

This study was conducted in the catchment basin of Lake Cuitzeo which is approximately 4000 km^2 in area. It is located in central Mexico between the north of Michoacán state and the south of Guanajuato state (Fig. 1). The climate is temperate with seasonal (summer) rainfall. Soils and landforms in most of the basin are derived from volcanic materials (lavas and pyroclasts). The land cover includes temperate forests, scrublands and agricultural lands. A large number of urban and rural settlements from twenty-eight municipalities occur in the study area (López et al., 2006; Mendoza et al., 2006). We have identified 16 oak species in this region, with *Quercus castanea* being the most common species (Aguilar-Romero et al., 2016).

Quercus castanea (section *Lobatae*) is a long-lived, moderately large tree reaching up to 18 m in height (Arizaga et al., 2009). This species is widely distributed from northern Mexico to Guatemala and El Salvador, usually found at elevations from 1180 to 2600 m (Valencia-Ávalos, 2004). At the study site, flowering occurs synchronously in the dry season from late March to early April, and fruiting from October to December. As other oaks, *Quercus castanea* is monoecious, producing separate male catkins and small female flowers, and it is an obligate outcrosser that is pollinated by wind. Acorns are dispersed mainly by gravity and birds such as acorn woodpeckers (*Melanerpes formicivorus*) and golden-fronted woodpeckers (*Melanerpes aurifrons*) (Schondube et al., 2010).

Leaves of 16 mature trees (i.e., >80 cm in diameter at breast height), separated at least 100 m from each other, were collected in each of four populations located at fragments of different size (Fig. 1, Table 1). At each of these four sites the density of *Q. castanea* trees was estimated with a 100 × 20 m transect. In general, trees show a regular spatial distribution in these fragments. Leaves from 16 spatially isolated trees (i.e., growing individually in agricultural or pasture lands and clearly separated from the nearest forest patch) distributed throughout the Cuitzeo basin were also collected. The average spatial distance between sampled trees was 14.6 km, with a minimum distance of 84.69 m (between the Ume fragment and isolated tree number 12) and a maximum distance of 69.5 km (between isolated trees 2 and 16).

From each of the eighty trees approximately 100 mature acorns were also sampled. Acorns were germinated and the first true leaves from ten seedlings per mother tree were harvested and stored at −80 °C until DNA extraction.

2.2. Microsatellite markers and genotyping

Genomic DNA was isolated from 100 mg of frozen leaf tissue using the protocol of Lefort and Douglas (1999). Seven nuclear microsatellite markers designed for *Q. rubra* (Aldrich et al., 2002) were chosen on the basis of reproducibility and polymorphism assessed in preliminary trials: *quruGA-2F05, quru-GA-0I01, quru-GA-1F07, quru-GA-0A01, quru-GA-1F02, quru-GA-0M07,* and *quru-GA-0C19*.

The multiplex amplifications were performed using the QIAGEN Multiplex Kit, (QIAGEN) in 5 μl reactions as follows: 1x multiplex PCR master mix, 2 μM of each primer and 5 ng of DNA. Forward primers were fluorescently labeled. The thermal cycling program