

# The Genetic Variability of *Quercus grisea* Liebm. in the Sierra Fria of Aguascalientes, Mexico

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## Introduction

*Quercus* L. (oaks, encinos, robles) is the largest genus in the *Fagaceae* with approximately 500 species (Rogers & Jonhson, 1998), mainly distributed in temperate and subtropical regions of the northern hemisphere (Nixon, 1998). Mexico is considered a center of diversity for the genus, with 150-200 species (Rzedowski, 1981; Nixon, 1998), of which approximately 115 are endemic (González-Rivera, 1993). As Mexico is a center of diversity for the genus, it is important to know the evolutionary phenomena that occurred within the populations in terms of population genetics and dynamic diversity. Genetic diversity is the most important factor on which adaptation and evolution depend and its maintenance is vital to the mechanisms of reproduction, cross-breeding and dispersal in a population or evolutionary lineage (Amos and Harwood, 1998).

Genetic diversity is measured by the use of markers, either morphological or molecular and among the great diversity of molecular markers that exist microsatellites should be emphasized as these, by their nature, show high variability between individuals. Microsatellites are repetitions of simple sequences from 2 to 10 pb, with a high rate of mutation and can be found in the DNA of the nucleus, mitochondria, chloroplasts and in other genetic material such as viruses, plasmids and transposons. Those of the nucleus show Mendelian inheritance and are co-dominant. Because of their capacity to differentiate between both individuals and large groups, microsatellites have been of great use in the analysis of parentage, allele distribution, genetic structure of populations and phylogenetic and phylogeographic studies (Hartl, 2000).

Identification of the level of variability of the species as well as the genetic dynamics of its population allows us to infer the state of conservation of the populations. High levels of variability are seen as healthy, conferring the capacity to respond to threats such as diseases, parasites, predators and environmental changes. Conversely, low levels of variability are seen as limiting the capacity of the species to respond to such threats in the short and long term (Amos and Harwood, 1998). The loss of genetic variability is associated with phenomena such as a reduction in population size, which can occur naturally due to several

factors (for example catastrophes, high predation or a reduction in the reproductive capacity of the species. However, in recent years the reductions in the population size is more associated with loss of habitat and over-exploitation of natural resources as a result of human activities (Frankham *et al.*, 2002).

In the Sierra Fria of Aguascalientes the oaks were subject to intense logging from 1920 to 1950 and suffered further from the development of extensive cattle ranches during second half of the 20<sup>th</sup> Century (SEDESO 1993; Minnich *et al.*, 1994). There are several areas in the Sierra Fria with serious problems of erosion where the oak woods are very disturbed.

The zone is composed principally of two types of vegetation: oak-pine forest (about 64%) and pasture, *Quercus resinosa* Liebm., *Q. potosina* Trel., *Q. grisea* Liebm. and *Q. eduardii* Trel. represent about 74% of the tree cover (Márquez-Olivas *et al.*, 2002). In 1995, this zone was declared as a zone subject to ecological conservation (ZSCE) and it was named by CONABIO as one of 153 terrestrial priority regions (RTP). Since then it has lost about 37% of the original vegetation (Arriaga *et al.*, 2000). Recent studies using aerial and satellite photographs have shown that from 1956 to 1993 the vegetation cover has been maintained, however, it was also shown that some human activity such as the creation of new agricultural lands and grassland has been responsible for some forest and grassland deterioration (Chapa-Bezanilla *et al.*, 2008).

*Quercus grisea* is one of the oaks that is abundant in the Sierra Fria forming forests on well-drained soils and is associated with juniper woods, oak woods and pasture. It is reported in three localities in two municipalities of Aguascalientes, one in the municipality of Calvillo and two in San José de Gracia (De la Cerda Lemus, 1997). The genetic study of this species can help us to understand the methods of dispersal that occur in the zone and to identify conservation zones in the Sierra Fria.

A great deal of work has been carried out in the world on the genetic diversity of oaks, including analysis of variability measured as an index, the detection of possible hybrids, phylogenetic analyses and phylogeographic studies, using a wide range of molecular markers from enzymes to sequence analysis. In the case of genetic studies on oaks using nuclear micro-satellites, contrasting results have been reported. Generally, genetic variability is found within the populations, which are not structured (Dodd & Kashani 2003; Dutech *et al.*, 2005; Muir, 2004). However, works exist in which clonal structure is reported, such as that of Ainsworth and collaborators in 2003. In Mexico, pioneering studies have been carried out on the genetic variability of the group and its population structure, particularly those of Alfonso-Corrado *et al.*, 2004, González-Rodríguez *et al.*, 2005 y González-Rodríguez *et al.*, 2006, in which the genetic variability and population structure of the analyzed species was measured.

Given the abundance of oak forests in the conservation zone of the Sierra Fria and that *Q. grisea* is one of the most abundant species, it is important to know the state of genetic conservation of their populations by evaluating the genetic variability as well as defining the populations that are more important in terms of gene flow. This will help us to understand more fully the mechanisms of pollination and dispersal that are common to oaks of the zone.

The objectives of this work are to estimate the level of genetic variability in the populations of *Q. grisea* in the Sierra Fria by the use of nuclear microsatellites, to identify the pattern of gene flow between populations and to define the populations that are most important genetically for ecological conservation.

As oaks are wind pollinated and long-lived, and as *Q. grisea* is an abundant species in the zone, it is hoped to find a high level of genetic diversity in terms of allele wealth. It is also hoped to find a high level of gene flow between neighbouring populations.

## Materials and Methods

During May and June 2007 leaves of 84 individuals of *Q. grisea* were collected from four sites in the Sierra Fria, namely “Rancho el Cepo” (CP), “Entrada a la Congoja” (EC), “Mesa el Sapo” (ES) and “Mesa Montoro” (MT). Leaves were collected from at least 20 individuals at each site. The descriptions of the sites and the individuals included are shown in Table 1.

The sites were surveyed and data entered into the program Arc View v. 3.1 in order to calculate geographic distances.

DNA was extracted from leaves of 84 individuals using the protocol DNeasy Plant Kit de QUIAGEN and one of its modifications. Fragments were amplified by PCR for the primers 1F02, OC19 y OC11 of the series quru AG (Aldrich *et al.*, 2002). The reactions took place in a volume of 25  $\mu$ l, 1X Taq Buffer, 10mmol dNTPs, 20mMol MgCl<sub>2</sub>, 0.0001 $\mu$ g/L de BSA, 10ng de ADN, 0.5 u Taq polymerase and 0.72 nm of each primer F and R. The amplification was carried out in a Thermocycler Gene Amp PCR System 9700 set at 94° C 3 min, (94° C 10 sec. (denaturation), Tm° C 10 sec. (alignment), 72° C 10 sec. (extension) X 30 (cycles) and 72° C 3 min (complementary amplification). The products were run in gels of 1% agar, 115 V for 40 min and visualised with Ethidium bromide (BrEt) exposed to UV light, using a 100 pb molecular marker as a reference. The amplification products were diluted 1/40 w/w and 1  $\mu$ l of the diluted amplified product was mixed with 9.75  $\mu$ l HiDi Formamide and 0.25  $\mu$ l ROX-500 or Rox400. The mixture was analysed in an ABI Prism 3700 (Applied Biosystems) using the method of analysis of fragments.

When the electropherograms were obtained, the size of each fragment was determined using the program Gene Scan Analyzer (Applied Biosystems). When the fragments had been identified, each one of the individuals was genotyped and the genotypes were entered into a database for genetic analysis. The calculations of expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), genetic distance and index of endogamy (Nei, 1972) were carried out using the program TFPGA. The calculation of genetic flow was made from the estimator theta ( $\Theta_{st}$ ) from paired data between populations. The estimation of exclusive and represented alleles was made by a direct count.

## Results

The loci OC19, 1F02 and OC11 were polymorphic for *Q. grisea* with variable sizes. Values are shown in Table 2. Genetically identical individuals were not found.

**Table 1.** Description of collecting sites.

Site	Municipio	Latitude N	Longitude W	Altitude m	Vegetation	Description	Number of individuals
Entrada a la congoja	San José de Gracia	22° 9' 39.4"	102° 32' 58.2"	2500	<i>Q. grisea</i> and <i>Juniperus depeana</i>	Flat pasture	20
Mesa Montoro	San José de Gracia	22° 00' 26.1"	102° 34' 25.6"	2384	<i>Q. grisea</i> and <i>Q. potosina</i> .	Flat with slight slope. Site used for cattle grazing	20
Mesa el Sapo	San José de Gracia	22° 14' 44.2"	102° 29' 31.4"	*	<i>Q. grisea</i> and <i>Q. potosina</i> .	Oakwood with flat areas, disturbed by cattle	21
El Cepo	San José de Gracia	22° 11' 06.9"	102° 35' 46.9"	2590	<i>Q. eduardii</i> , <i>Q. grisea</i> and <i>Q. potosina</i> .	Oakwood, slightly disturbed inside the hunting area	23

**Table 3.** Number of alleles, exclusive (E) and observed (O) for each locus per population.

Site	OC19		1F02		OC11		Total
	E	O	E	O	E	O	
CP	1	12	0	11	2	10	33
EC	6	17	0	10	1	10	37
ES	6	15	2	13	1	11	39
MT	6	12	1	12	1	10	34

**Table 4.** Heterozygosity expected (Ho) and observed (He) per allele and population for *Q. grisea*.

Site	OC19		1F02		OC11		Total
	Ho	He	Ho	He	Ho	He	
CP	0.5217	0.8483	0.6087	0.8048	0.9565	0.7971	0.8167
EC	0.4000	0.9244	0.6842	0.8549	0.8421	0.8634	0.8809
ES	0.4286	0.9338	0.6667	0.9024	0.9048	0.8676	0.9013
MT	0.5500	0.8282	0.7222	0.9302	0.8500	0.7397	0.7397
Average	0.4762	0.9181	0.6667	0.8886	0.8916	0.8255	0.8774

**Table 2.** Relationship of fragments for the series *quru* GA in *Q. grisea*.

<i>quru</i> GA	OC19	1F02	OC11
No of individuals	84	83	82
No of loci.	31	17	15
Range in size.	198-382	140-242	190-224

The primer OC11 showed the highest value for a polymorphic locus compared to those reported in previous studies.

The number of alleles present in each population per locus per site and the number of exclusive alleles was variable between the populations with “Mesa el Sapo” showing the highest number of represented alleles. The data are summarised in Table 3.

The observed heterozygosity for all populations shows a high (0.6781) level of genetic diversity and corresponds to obligate outcrossing organisms. For the loci OC19 and 1F02 it can be seen that there is a deficiency of heterozygosity, in the case of allele OC11, it can be seen that the frequencies are in equilibrium. At the population level, the analysis is different, in that the 4 populations are very close to equilibrium between homozygosity and heterozygosity. The populations closest to equilibrium are CP and MT (Table 4).

The estimated coefficient of endogamy for all loci for all populations ( $\Theta_{st}$ ) is 0.0265, which is a low value and shows that the populations are not genetically structured and share gene flow between populations. This tells us that in genetic terms, the populations are very similar, that selection and endogamy are not occurring and the mechanisms controlling the genetic diversity between the populations, such as dispersal and pollination, are effective. From these data it can be deduced that the most similar populations are EC and ES, while the most different are CP and MT (Table 5).

In terms of genetic distance the populations CP, EC, and ES are statistically very similar, with MT the most different, as well as the population most distant from the others. This tells us that distance may be a factor in the genetic isolation of population MT. For the other populations distance is not a factor that causes isolation as the most similar populations are not the closest (Table 5).

Gene flow between populations tells us that it is greatest for populations EC and ES, which correlates with the genetic distance, i.e. what maintains the identity of each population are the mechanisms of dispersal and pollination (Table 5).

**Table 5.** Comparison of distance, geographic ( $D_{geo}$ ), genetic ( $D_{gen}$ ), coefficient of endogamy ( $\Theta_{st}$ ) and gene flow ( $N_e m$ ).

	$D_{geo}$	$D_{gen}$	$\Theta_{st}$	$N_e m$
CP vs EC	5.61	0.2902	0.0237	10.30
CP vs ES	12.59	0.2901	0.0234	10.43
CP vs MT	19.81	0.4055	0.0487	4.88
EC vs ES	11.12	0.2905	0.0057	43.61
EC vs MT	17.23	0.4514	0.038	6.33
ES vs MT	27.95	0.3116	0.0198	12.38

## Discussion

The observed genetic variability measured in terms of wealth of alleles shows that *Q. grisea* has a broad genetic variability. The locus *quru* AG OC19 showed a greater variability for an allele than the previously reported range of 10-14 loci. This shows that the marker is very good for studies of genetic variability and that the allelic diversity in the species is very high.

As all the genotypes proved to be different from each other it can be inferred that reproduction in *Q. grisea* is preferably sexual and by outcrossing. We did not sample from individuals close to one another, as we would expect to find genetically identical individuals because of the low number of loci used.

In the populations of “Mesa de Sapo” and “Entrada a la Congoja” were found most indications of allelic wealth, nevertheless the population of “Mesa Montoro” also has a high amount of exclusive alleles. The neutral theory in genetics of populations predicts that the greater amount of alleles is in populations of great size with ancestral distribution, indicating that these three populations are the candidates to be oldest and greatest in population size. The population of “El Cepo” could be the result of a recent colonisation. In order to verify this hypothesis, further studies of phylogeography by analysing sequences of the populations can generate demographic studies for each of the populations. It is possible to indicate that the three sites with greater allelic wealth are also associated with plains, oakwoods or pastures. This can be because the reproductive success in plains generates a high amount of individuals with the corresponding generation of new alleles by generation.

The heterozygosity data show that the populations are closely balanced between homozygosity and heterozygosity, and it can be deduced that at the interior of the populations phenomena of natural selection or intense processes of genetic drift or endogamy are not occurring. Nevertheless it was observed that loci OC19 and 1F02 show that there is a slight deficiency of heterozygosity that could denote a selection process, which it is being concealed by the heterozygosity of allele OC11. Although in general terms we cannot speak of a selection process among the heterozygotes, the deficiency of heterozygotes can be considered to be the result of a reproductive process such as endogamy acting on the populations. Another explanation is that the expected heterozygosity depends as much on

the amount of alleles as their frequencies. If a high amount of alleles with low allelic frequency occurs, the index of expected heterozygosity should be high and as the population size used for the study is small it is possible to underestimate the amount of heterozygosity in the population because their frequencies are not equalled. Although the expected heterozygosity can be overestimated, the observed heterozygosity is a good estimator to identify the genetic variability, the average value for all the populations corresponds to the organisms with sexual reproduction and obligate outcrossing and is very similar to the values reported in previous studies for oaks.

The coefficient of endogamy is very low (0.0265) and shows that the populations are not structured genetically and are very similar to each other. This may be because wind can be very effective for pollination and that the pollination strategy of *Q. grisea* allows the exchange of gametes over large distances which causes the genetic maintenance of the species in the zone.

The paired data for genetic distance as well as endogamy coefficient are very similar between the populations. The populations of “El Cepo”, “Entrada a la Congoja” and “Mesa el Sapo” in terms of distance, form a single group whereas “Mesa Montoro” is the



*Quercus grisea*

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most isolated population. The intensity of gene flow between “El Cepo”, “Entrada a la Congoja” and “Mesa el Sapo” may be causing its identity in genetic terms and large geographic distance may be a factor that limits the genetic exchange between populations. If oaks are pollinated by wind then it can be deduced that the effectiveness of wind as a pollinator is reduced when distances are great. Another factor that can alter the genetic flow between the populations is the presence of geographic barriers, and although “El Cepo” and “Entrada a la Congoja” are the closest populations to each other, they are not the most similar genetically. “Entrada a la Congoja” and “Mesa el Sapo” are the two most similar populations, probably because there are no mountainous barriers between these two and the distance between them is not great as it is between them and “Mesa Montoro”. It therefore appears that the main obstacle for wind pollination is firstly geographic barriers and secondly geographic distance.

In terms of conservation the population that meets the requirements of high allelic wealth, presence of exclusive characters and a high level of gene flow is

“Mesa el Sapo”. However, if conservation efforts are directed solely to this one population there is the risk of losing exclusive alleles in the long term, as can also occur at “Mesa Montoro”, the population with greater isolation in terms of gene flow. For this reason it is recommended that conservation efforts be concentrated on the populations of “Mesa Montoro” and “Mesa el Sapo” to conserve the greater allelic wealth as well as the greater amount of exclusive alleles. If the intensity of gene flow remains as it is today, the population of “Entrada a la Congoja” can remain as a corridor of alleles between both populations. It is likely that data related to population demography would be needed to plan the long-term viability of each of the populations.

A recent study has quantified the changes of ground use in the zone by analysis of aerial photos and of satellite images which show that from 1956 to 1993 the forest been neither reduced nor increased, that is to say the cover stays constant. Nevertheless, the same study also states that in terms of degradation of habitat in the Sierra Fria of Aguascalientes there is a tendency for the forest to increase (Chapa-Bezanilla *et al.*, 2008). This is very important for the establishment of strategies of conservation in the zone. The “Entrada a la Congoja” is the population that has the greater risk of disappearing, as the study considers that there is a growth of induced cattle pastures that can affect population growth.

## Conclusions

The genetic variability of *Q. grisea* in the Sierra Fria of Aguascalientes, in terms of allelic diversity, is high.

Evolutionary processes that act on the genetic diversity of the populations of *Q. grisea* in the Sierra Fria were not detected.

The populations of *Q. grisea* are not genetically structured.

The populations of “El Cepo”, “Entrada a la Congoja” and “Mesa el sapo” can be considered in genetic terms as a single group, with the population of “Mesa Montoro” the most different genetically.

In the Sierra Fria, the processes of pollination and dispersal are very effective in maintaining genetic diversity.

In terms of conservation, the most important population is “Mesa el Sapo”.

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