

# Effects of the geographical scale in hybrid detection at extensive contact zones between *Quercus faginea* and *Q. pyrenaica*



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## 1 - Background

The prevalence of hybridization in trees at a global scale is already well-known. Oaks are among the most widely represented trees in the Iberian Peninsula and represent model systems of natural introgressive hybridization. Predicting the evolution of forest stands in the current climate change scenario requires previous identification of individuals of presumable hybrid origin, as well as of leaf traits that may allow their recognition. The main aim of this study is to **highlight the importance of considering the effects of scale and the likely presence of Isolation By Distance (IBD) during the selection of study plots**, within the context of research intended to assess the **implications of leaf trait responses of hybrids and their parents to environmental change**. With this objective, two oak species (*Quercus faginea* Lam. and *Quercus pyrenaica* Willd.) and their hybrids were sampled across geographic and environmentally distinct contact areas and the levels of hybridization were determined. Then, IBD was evaluated using Mantel tests and the effects of scale were analyzed through Spatial Autocorrelation Analysis in order to perform an adequate selection of a study scale.

## 2 - Methods

### Study area and sampling design

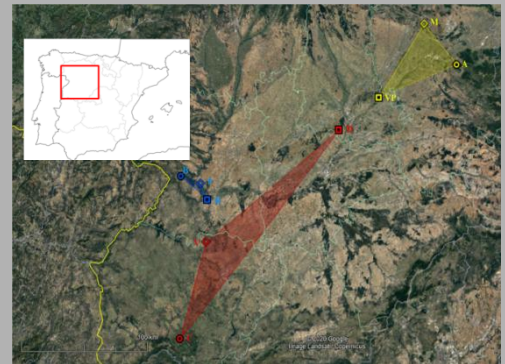
Three plots each representing either an apparent monospecific assembly of one of the parental species or of hybrid individuals, distributed at three different geographic scales were selected: fine scale (plots B, P, H; maximum distance ca. 40 km) / medium scale (plots A, M, VP; maximum distance ca. 100 km) / large scale (plots, C, V, D; maximum distance ca. 200 km). Mature leaves were collected from thirty individuals within each plot.

### Genetic classification of the individuals

The individuals were genotyped through their AFLPs profiles. Bayesian Analysis of Population Structure (software STRUCTURE) and Multivariate Analysis (Principal Coordinates Analysis, PCoA) were used to investigate genetic structure in the three plots within each scale; first, separately and, second, considering the nine plots altogether. In absence of IBD, a very similar classification of the individuals should be expected. Three genetic categories (two of them corresponding to "pure" species and a "hybrid" one) were established based on overall genetic composition.

### Evidences of isolation by distance

IBD was tested through Mantel tests and Spatial Autocorrelation Analysis. Both analyses were performed taking the nine described plots altogether, for each scale separately and combining scales by pairs.



Location of the nine study plots. Scales are represented with colors: large in red, medium in yellow and fine scale in blue. Symbols show the dominant "general morphology" of each plot: circles for *Quercus pyrenaica*, squares for *Quercus faginea* and diamonds for hybrids.

## 3 - Results

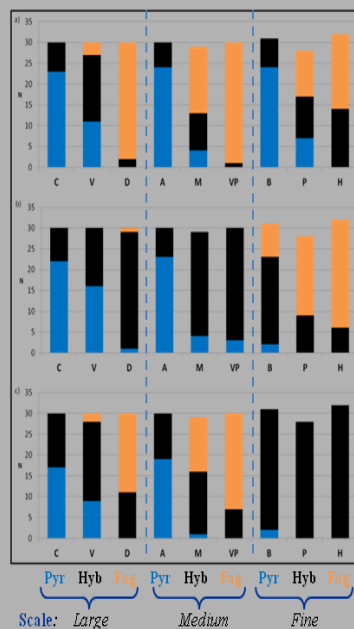
### Structure and genetic categorization of the populations

*a) Scales analysed separately:* plots dominated by "pure" species included that species and some hybrids, but not the other "pure" species. In the case of "hybrid plots", they included individuals from the three genetic categories.

*b) Scales analysed altogether; K=2 (STRUCTURE):* similar results for some plots (C, A, H), as compared with the previous analysis (a). Results slightly different for other plots (V, M, P), and very different for the rest of them (D, VP, B).

*c) Scales analysed altogether; K=3 (STRUCTURE):* results clearly different, especially at a fine scale. A third cluster that includes the geographically closest individuals is identified.

Summing up, a strong disparity in the classification of individuals is observed between those analyses that consider the three scales separately and those referred to the whole data set. The problem arises when the individuals belonging to the "fine scale group" are included in the analysis. This fact, together with the observed agreement between Bayesian and Multivariate analysis (PCoA) suggests a likely effect of IBD.

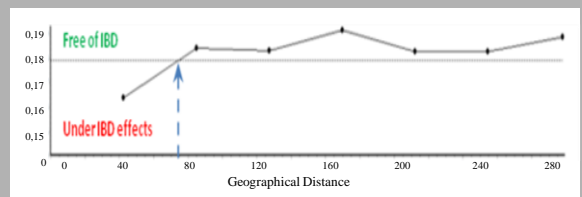


### Analysis of the distance effects

Mantel tests were statistically significant in all cases, except for that performed for the large scale separately, which confirms an effect of IBD in those plots included in the fine and medium scales.

Scales	r	p
Fine + Medium + Large	0,241	0,001*
Fine	0,263	0,001*
Medium	0,212	0,001*
Large	-0,004	0,556
Fine + Medium	0,344	0,001*
Fine + Large	0,427	0,001*
Medium + Large	0,156	0,001*

Spatial Autocorrelation Analyses confirmed that the average genetic distance between pairs of individuals is smaller than random expectations when individuals are separated by distances <65 km.



Correlogram obtained from spatial autocorrelation analysis carried out for all plots. Each distance class is defined by a geographical range (in km) represented in x-axis.  $A_s$ -value is shown in y-axis. The dotted line points out the average  $A_s$ -value of the all data set, distance classes above that value are free of IBD and the ones which are below are under IBD effects.

## 4 - Conclusions

IBD strongly affects to those individuals from our data set separated by ca. less than 65 km, which could be due to differences in pollination and dispersal rates, but further studies are needed to confirm this in the studied species. In addition, the **limitations of selecting study-plots at different scales** should be borne in mind. In order to overcome these limitations there is a necessity of developing **methodological improvements** in studies aimed at assessing responses of oaks to environmental changes along geographic gradients.