Hybridization Processes in Mediterranean Oaks from South Spain

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This work presents the results from a study of hybridization processes in an area disturbed by man. Five different species of genus *Quercus* coexist in this area (*Q. coccifera* L, *Q. faginea* subsp. *broteroi* (Coutinbo) A. Camus, *Q. lusitanica* Lam., *Q. rotundifolia and Q. suber* L.).

In these areas, most of the possible hybrids among the species mentioned before have been found. However, our study focused on those hybrids in which *Q. coccifera* is a parent (*Q. coccifera* x *Q. faginea subsp. broteroi; Q. coccifera* x *Q. lusitanica; Q. coccifera* x *Q. rotundifolia* and *Q. coccifera* x *Q. suber*).

The results have made it possible to evaluate how hybridization processes are concentrated in contact areas between woods and cultivated lands, called Tension Areas. The structure of the hybridization areas correspond to the Mosaic model. The study of the factors which contribute to the hybridization processes in the area show the following results:

1) There is a clear relationship between the number of hybrids and the population of parental species available, as well as between the number of hybrids and the size of the disturbed area.
2) Hybrids have less viability in their pollen grains than parental species.

3) there is a gradient in the viability of pollen grains from parental species, with greater viability in pollen grains from specimens in contact with the hybridization areas than in those which are distant from these areas.

From the results it can be concluded that diversity at the site clearly contributes to the hybridization processes, although there are physiological barriers which slow down the expansion of hybrids in the areas occupied by the species.

Introduction

Hybridization processes in the *Quercus* genus are studied throughout the world (Huguet del Villar, 1935; Hardin, 1975; Hy, 1895, Nixon and Muller, 1993; Palmer, 1948; Jucker, 1952). The problems in the identification of numerous specimens belonging to the genus *Quercus* due to the hybridization of particular species are well known. Introgression problems make the identification task even more difficult, as reported in numerous works (Burger, 1975; Coombes, 2000; Cousens, 1963; Lamant and Sternberg, 2000; Potter, 1994).

In the South-west of the Iberian Peninsula it is possible to identify the presence of at least eight species of the genus *Quercus* L (*Q. canariensis* Willd., *Q. coccifera* L., *Q faginea* Lam., *Q. lusitanica* Lam., *Q pyrenaica* Willd., *Q. robur* L., *Q. rotundifolia* Lam. and *Q. suber* L.). These species occur in numerous enclaves where the weather, edaphic and management conditions make possible their coexistence. Hybrids are easily found in some areas where several species of this genus appear (Vázquez, 1995; Vicioso, 1950).

It has been shown in different studies that the structure of the woods and disturbance may promote hybridization processes (Cruzan and Arnold, 1993; Freeman et al., 1995). In the woods of the South-west Iberian Peninsula, *Quercus* are frequently disturbed by man and occasionally by natural factors such as the fire. Under such conditions, hybridization processes have been studied in populations in which five taxa of the *Quercus* genus coexist (*Q. coccifera* L. *Q. faginea* subsp. *broteroi* (Coutinho) A. Camus, *Q. lusitanica* Lam., *Q rotundifolia* Lam. and *Q. suber* L.). These taxa have been strongly disturbed by the destruction of the woods, in order to use the land for farming or stockbreeding. This work has focused on determining some of the pre-embryonic barriers within the parental species and hybrids that slow down hybridization or introgression processes in the population.

Material and methods

Study area - The area under study is a zone with a great diversity of *Quercus* species. This area is near the Valley of Santa Ana, in the south of Badajoz province (Spain) (U.T.M. 29SPD94), 650 m. above the sea level, 800 mm/m^2 of the annual precipitation, 13,4ºC annual mean temperature and clay soil.

In this area it is possible to find at least five different *Quercus* species. Human disturbance, including cultivation with trees (olive), scrubs (wine) and grasslands (oats and barley), has been an element of the disturbance in the areas.

Hybridization area - The studied specimens, including their distribution, abundance, mass and size of disturbed surface areas, have been calculated with a GIS, Pro XR model, Trimble, with software incorporated (Pathfinder 2.11) This has made it possible to draw the maps of the studied areas and of the distances between points (Trimble, 1998).

Once the study of each species and hybrid was finished, the study of the hybridization area took place, focusing on the models suggested by authors such as Arnold, (1993 and 1997); Harrison, (1986 and 1990) and Howard (1986). To reach the main aim, the distribution of the number of hybrids was studied from the known maximum distance in the woods of parental

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species (excluding *Q. coccifera*). The study of the hybridization area structure is supplemented with the graphic representation of the information provided by the GIS, and the statistical analysis of the data through the mean tendency line (Snedecor and Cochran, 1980).

Apart from knowing the hybridization area, the relation between the number of hybrids and the available parental surface has been studied (the structure and density of the parental masses is the same for all the studied species, excluding *Q. coccifera* L., 40-45 feet/ha and mean age of 100-150 years old). The number of hybrids and disturbed surface areas available has also been studied. Finally, the combination of these two factors previously mentioned has also been studied (parental surface plus disturbed surface) versus the number of hybrids found.

The statistical analyses used in this study have been lineal regressions between the studied parameters (Snedecor and Cochran, 1980).

Hybrid studies - Together with the *Quercus* species, we also found different hybrids, including the following: *Q. coccifera* x *Q. rotundifolia; Q. coccifera* x *Q. suber; Q. coccifera* x *Q. lusitanica; Q. coccifera* x *Q. faginea* subsp. *broteroi; Q. faginea* subsp. *broteroi* x *Q. lusitanica; Q. faginea* subsp. *broteroi* x *Q. rotundifolia; Q. faginea* subsp. *broteroi* x *Q. suber; Q. lusitanica* x *Q. suber* and *Q. rotundifolia* x *Q suber*.

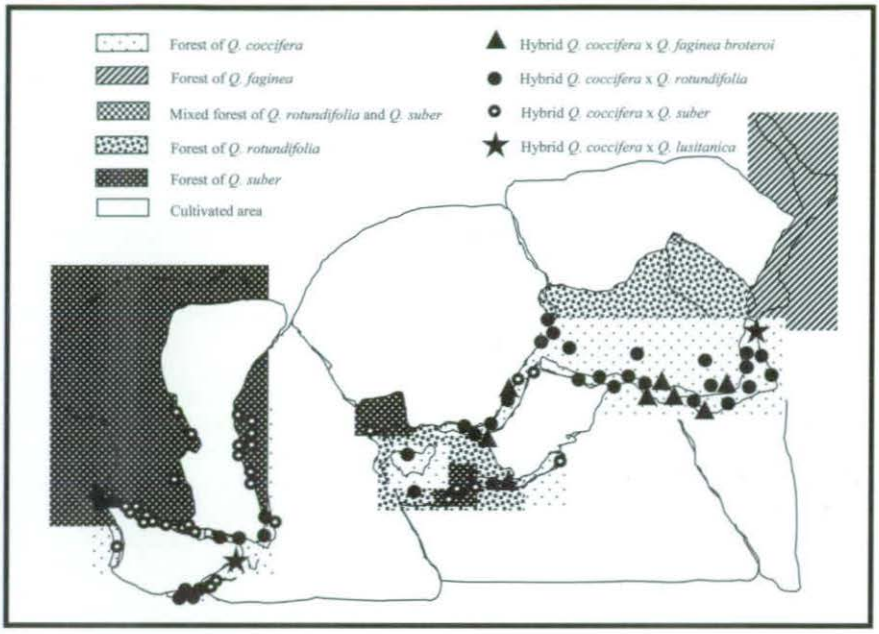
The differentiation between the different hybrids and their parents has been established according to morphological characteristics, such as the leaf, style, cupule, male flower and acorn morphology. Samples of the material studied were conserved in the herbarium of the Investigation and Technological Service (HSIA), with the numbers 4268 up to 4325.

There was difficulty differentiating and completing the study of all the hybrids . That is why we selected only the hybrids of *Q. coccifera*. These taxa have stable leaf characteristics (limb morphology, petiole, presence of hairs and marginal spines) and a clear period of flowering. Other taxa, such as *Q. faginea* subsp. *broteroi, Q. lusitanica* or *Q. rotundifolia* have many variations in leaf morphology, pubescence and margin, and introgression processes are frequent. The small number of the individuals of

Table 1. *Distribution surface and number of hybrids (only with* Q. coccifera*) inside the studied area.*

Taxa	Surface occupation (ha)	Number individuals
Q. coccifera	*1,65*	*uncountable*
Q. faginea subsp. *broteroi*	0,76	*55*
Q. lusitanica	*0,01*	*8*
Q. rotundifolia	*6,28*	*uncountable*
Q. suber	*9,38*	*uncountable*
Q. coccifera x *Q. faginea broteroi*	—	*11*
Q. coccifera x *Q. lusitanica*	—	*2*
Q. coccifera x *Q. rotundifolia*	—	*36*
Q. coccifera x *Q. suber*	—	*29*

Figure 1. *Distribution of the different forest species and hybrids found in the study area. Scale 1: 250000. (see legend)*



Q. *lusitanica* and its hybrids (*Q. coccifera* x *Q. lusitanica*) were also excluded.

Study of pollen - Together with the possible phenological barriers, which might be present among the species involved in the study, there are others pre-embryonic barriers which determine the crossing possibility among individuals of different species of the genus *Quercus*. The viability and fertility of the pollen grains are elements that determine the individual's competitiveness inside the populations to transfer their genes to new individuals and facilitate crossing processes between species and /or hybrids.

The viability of pollen grains has been studied using the fluorescein diacetate technique (Heslop-Harrinson and Heslop-Harrison, 1970) In this case, the possible relation between the fertility of the pollen grains and the distance to the disturbed area of the masses was demonstrated.

The statistical analyses used in this case was lineal regressions among the studied parameters and ANOVA analysis contrasting means using Turkey's method ($p<0,01$), for the different studied taxa (Snedecor and Cochran, 1980).

Results

The following results have been derived from the study of the area of each species and hybrid (The position and direction, of the results expressed in table 5, appear in Figure 1)

In Figure 1 it can be seen that there are three areas clearly differentiated by the types of hybrids: In the eastern side there are mainly hybrids of *Q. rotundifolia* and *Q. coccifera*. In

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the western side the most common hybrids are those between *Q. suber* and *Q.coccifera*; and in the centre of the subject area, there are hybrids among all the species involved in the study, except *Q. lusitanica*.

Hybridization area type

The results represented in Figure 2 were obtained from the study of the structure in the hybridization area for each parental species involved in the study.

The results expressed in figures 2a, 2b, 2c and 2d, show that in all the cases the hybridization are a mosaic model (Arnold, 1997).

There are hybridization areas associated with the copse isles left by man, alternated with cultivated areas. Hybridization has taken place in most of the study area. It has not been concentrated in a particular area.

Hybridization area analysis

As indicated in the material and methods section, apart from the study of the hybridization area structure, the relationships between the total number and the parental surface available were analyzed; as well as the surface in contact with farming areas (disturbed areas). The results appear in figures 3, 4 and 5.

Figure 2. *Distribution frequency graphics of different hybrids dependent on the distance in the parental forests, for the hybrids a:* Q. coccifera x Q. suber, *b:* Q. coccifera x Q. rotundifolia; *c:* Q. coccifera x Q. faginea *subsp.* broteroi *and d:* Q. coccifera x Q. lusitanica.

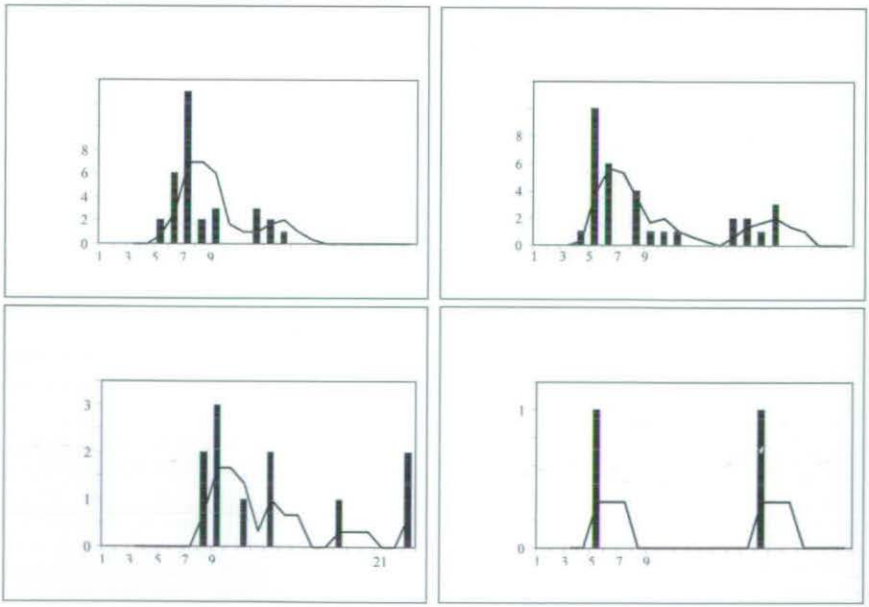


Figure 3. Lineal regression between the number of hybrids (*Q. coccifera* x *Q. suber*, *Q. coccifera* x *Q. rotundifolia*, *Q. coccifera* x *Q. faginea* subsp. *broteroi* and *Q. coccifera* x *Q. lusitanica*) and the surface occupied by their parents (*Q. faginea* subsp. *broteroi*, *Q. lusitanica*, *Q. rotundifolia* and *Q. suber*).

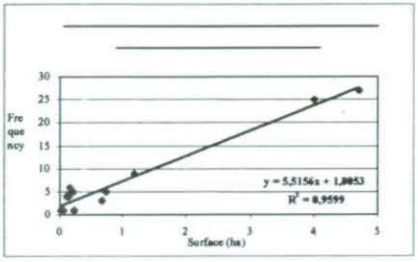
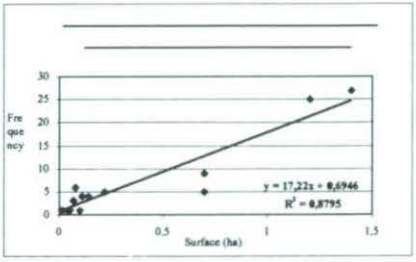


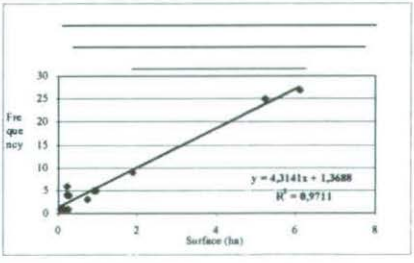
Figure 4. Lineal regression between the number of hybrids (*Q. coccifera* x *Q. suber*, *Q. coccifera* x *Q. rotundifolia*, *Q. coccifera* x *Q. faginea* subsp. *broteroi* and *Q. coccifera* x *Q. lusitanica*) and the disturbed surface available in the forest of their parents (*Q. faginea* subsp. *broteroi*, *Q. lusitanica*, *Q. rotundifolia* and *Q. suber*).



Finally the results found by comparing the sum of the surface occupied by the parents species plus the area in contact with the cultivated lands present in the small woods of the parental species are presented.

The results expressed in the three previous figures show that there are clear relations between the number of hybrids and the parental surface available (r^2 =0,95), as well as between the number of hybrids and the disturbed area (r^2= 0,87). However, the biggest relation (r2= 0,97) is found by adding both effects: the surface occupied by the parental species plus the disturbed area.

Figure 5. Lineal regression between the number of hybrids (*Q. coccifera* x *Q. suber*, *Q. coccifera* x *Q. rotundifolia*, *Q. coccifera* x *Q. faginea* subsp. *broteroi* and *Q. coccifera* x *Q. lusitanica*) and the disturbed surface available in the forest plus the surface occupied by their parents (*Q. faginea* subsp. *broteroi*, *Q. lusitanica*, *Q. rotundifolia* and *Q. suber*).



Pollen quality and hybridization process

Together with the study of the hybridization area structure and its implications in the processes which contribute or slow down the appearance of hybrids, one of the barriers which clearly limit the hybrids formation was also studied: the viability of pollen grains.

The global study of the viability of pollen grains in parental species and hybrids has provided the results expressed in Table 2.

The results expressed in table 2 show that there are clear differences between the viability of pollen grains from the parental species and those from hybrids. The parents species present higher viability than those from the hybrid specimens. Regarding the parent species, differences were found. Pollen grains of *Q. coccifera* had high viability (98,88), while those of *Q. faginea* subsp. *broteroi* had moderate viability (77,53).

Once the viability of pollen grains and the hybridization area structure were known, the viability of pollen grains of the parental spe

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cies was correlated with the distance to the disturbed areas or hybridization areas. The results found in four of the parents species are represented in figure 6 (*Q. lusitanica* has been excluded since there was a reduced sample of this specimen).

The results expressed in figures 6a, 6b, 6c and 6d, show, in all the cases, a tendency for an increase in the viability of pollen grains as one gets nearer to the areas disturbed by man, or to hybridization areas.

In the cases of *Q. rotundifolia*, *Q. coccifera* and *Q. suber* there are very clear relationships, whereas *Q. faginea* subsp. *broteroi* presents values for the r^2 of the regression are under 50% (r^2= 0,40).

Discussion and conclusions.

The study of the hybridization area structure (area with the greatest quantity of hybrids) has coincided with the areas in contact with the small woods of the *Quercus* genus and the cultivated lands. These results coincide with the ones found in other studies such as the Lenz's (1959) for populations of the *Iris* genus or Urbanska and Landdolt (1999) for *Cardamine* in cultivated lands. The disturbance and diversification of the environment in a habitat fosters hybrids when there are at least two species able to cross. The hybrid individuals are usually the ones which occupy the new areas successfully, since they have a more varied genetic heritage than the parents species from which they came.

The particular study of the structure on which the hybridization processes are organized in the studied area has let us know that it corresponds to the "Mosaic" model purposed by Barton & Hewit (1985). These results let us to conclude that, on the whole study, there are the same possibilities of producing hybrids. There would not be barriers in the crossing between species, or geographically in certain areas. These results could be useful to confirm that there would not be considerable pre-pollination or post-pollination barriers between species, and particularly between *Q. coccifera* and the rest of the species. These results would confirm again the previous contributions mentioned out in the introduction about the easy tendency existing in *Quercus* for hybridization (Hardin, 1975; Hy, 1895; Nixon & Muller, 1993; Palmer, 1948; Tomlinson et al., 2000).

The specific study of the causes which makes possible the hybridization process in the study area has provided us with some interesting results to understand the hybridization process in these species. There is a clear relationship between the disturbed area surface, the surface occupied by parental species (there is the same density of trees per unit in all the species crossed with *Q. coccifera*) and the number of hybrids produced. These results are mostly consistent with the ones exposed in Anderson's theory (1948) for hybrid habitats. Due to man's management, some different and interrelated niches have been created. These differ from the original habitats occupied by species. This new situation contributes to the presence and perpetuation of hybrids in all the areas under study. Examples like those found in our study have previously been published for other genera such as *Rubus* (Wiegand, 1935), *Iris* (Lenz (1959), *Salvia* (Grant & Grant, 1964) or *Cardamine* (Urbanska & Landolt (1999).

The model reflected in the results commented on beforehand, fits the model of the Tension Area (Arnold, 1997; Barton, 1989). This model,

Table 2. Viability pollen for fluorescein diacetate technique (Heslop-Harrison & Heslop-Harrison, 1970) in the parent species and hybrids (Q. co. x brot.: *Q. coccifera* x *Q. faginea* subsp. *broteroi*; Q. co. x suber: *Q. coccifera* x *Q. suber*: *Q. coccifera* x *Q. suber* and Q. co. x Q. rot.: *Q. coccifera* x *Q. rotundifolia*). a, b, c; indicate significant differences (ANOVA, Turkey; p <0,001). N: number of individuals studied.

Q. coccifera	*Q. f. broteroi*	*Q. suber*	*Q. rotundifolia*	*Q. co.* X *Q. brot.*	*Q. co.* X *Q. suber*	*Q. co.* X *Q. rot.*
n= 22	n= 12	n= 8	n= 12	n= 16	n= 12	n= 18
96,88±3.63 a	77,53±15.17 b	91,64±5.91 ab	93,35±5.24 ab	44,70±8.16 c	35,01±24.55 c	50,86±7.63 c

where species of the same genus are found living in plant or animal populations, is favorable for hybridization processes and others processes indirectly linked to hybridization when these species are disturbed by different phenomena (man, fire, avalanche etc.). The Tension Areas in our case would coincide with the areas named disturbed areas, hybridization areas, or areas in contact with woods and cultivated lands.

The last results did not offer a favorable view for the hybrid specimens in the area under study. There were all the conditions to stay in permanent niches without trees and even they could have competed with parental species. However, the space distribution in Figure 1 does not offer this view. The hybrids gather in the areas in contact with the small woods and the cultivated lands (Tension Area). The analysis of the viability of pollen grains for the studied parental species and hybrids provides us a new perspective of hybridization processes produced in the area under study. The hybrids show a viability considerably lower than the viability of pollen

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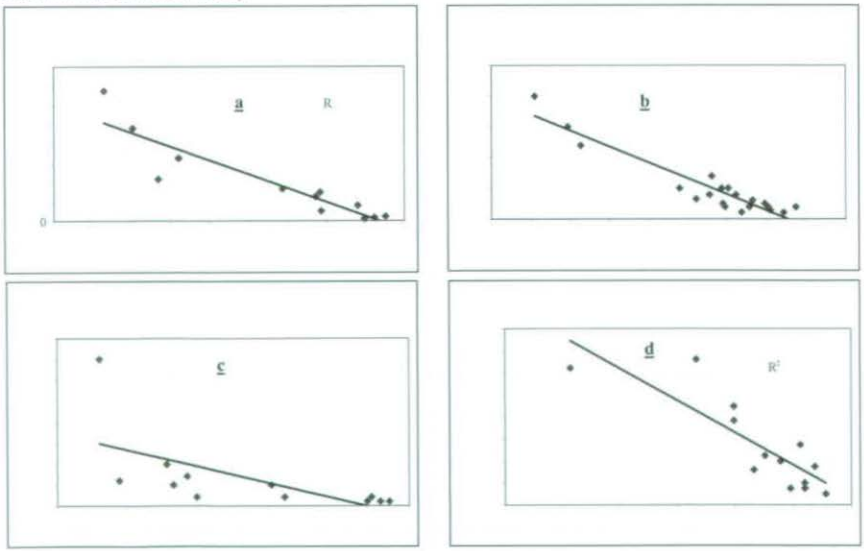
Figure 6. *Pollen grain viability in the different parents species (a:* Q. rotundifolia, *b:* Q. coccifera, *c:* Q. faginea *subsp.* broteroi *and d:* Q. suber*) and relationships with the distance to hybridization area (or contact area with the cultivated lands)*



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grains of the parental species. For some hybrids (*Q. coccifera* x *Q. suber*) the viability is under 10%. These results demonstrate the limitations of hybrids to transfer their genes, since they have less reproductive possibilities than the species. These data would partly coincide with Muller's (1952), and they would justify the hybridization processes in the *Quercus* genus as a necessary mechanism for the evolution of the species.

The last study showed the tendency of all the studied species (except *Q. lusitanica* and included *Q. coccifera*), to increase the viability of the pollen grains in the specimens close to the Tension Area. It is a mechanism favorable for the competence among the species to assure their genetic heritage. This behavior has been noticed in other tree genera such as *Pinus* (Clausen 1965) or *Eucalyptus* (Ladiges,1976). According to certain authors like Grant (1989), Arnold (1997) or Urbanska & Landolt (1999) in all plant and animal populations it is possible to find differences in the reproductive behavior of some local breeds. These differences are associated with environmental characteristics of their niche. In our case, the area called Tension Area would be favorable for the viability of pollen grains in the parental species, versus the hybrid specimens, slowing down even more the crossing possibilities of the hybrid specimens versus the parental ones.

Finally we can conclude that the process of hybridization is promoted in the Tension Zone, but only between the parent species, because reproductive barriers already exist in the hybrids.

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