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# A hypothesis on the evolution of isoprenoid emission by oaks based on the correlation between emission type and *Quercus* taxonomy

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**Abstract** We show that Mediterranean oaks that emit isoprene, monoterpenes or no isoprenoids belong to different subgenera as indicated by morpho-taxonomy and molecular genetics. On the other hand, oaks from North America and Asia that are taxonomically similar to the Mediterranean monoterpene emitter *Q. ilex* emit isoprene only. We surmise that isoprene emission is a genetic character which evolved ancestrally in the oak genus since this is the prevalent emission type in oaks widespread around the world and adapted to different environments. This ancestral character may have been either lost or modified in more recent clades such as those originating the Mediterranean oaks. If our hypothesis is correct then the taxonomy of European oaks is validated by this independent trait. Isoprenoid emission could serve as a chemo-taxonomical marker and could be used to reconstruct the phylogeny of oaks in association with molecular markers.

**Key words** Chemo-taxonomy · Isoprenoid emission · Evolution · *Quercus*

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## Introduction

Oaks (*Quercus* spp.) are reported to emit isoprene (Loreto and Sharkey 1990) or monoterpenes (Loreto et al. 1996) derived from recently fixed photosynthetic carbon. The emission type is species-specific and is not controlled by environmental conditions. The reason why the emission occurs is currently debated. One of the most interesting hypotheses is that isoprene and monoterpenes stabilize membranes and protect leaves against high-temperature damage (Sharkey and Singsaas 1995). This suggests that isoprenoid formation is an adaptive character associated with the establishment of oak species in harsh environments.

Alternatively, it is possible that the emission of isoprenoids is a genetic character which evolved anciently and is independent of environmental adaptation. The genetics of oaks are controversial because oaks are among the oldest plants that colonized the boreal hemisphere and oak species have a widespread area of distribution and a high degree of interspecific hybridization. Isoprenoid emission may be a useful marker of genetic distances between oak subgenera and species.

To gain understanding about the link between isoprenoid emission and genetic characters, we have compared the isoprenoid emission from oak species growing in Mediterranean forests and taxonomically classified in different subgenera on the basis of morpho-anatomical characteristics and of genetic differences. We also compared the emission of Mediterranean species with that of species that have morphological affinities with the Mediterranean monoterpene emitters but grow in different continents and environmental conditions. We report available data on a North American species (*Q. agrifolia*), and also, for the first time, surveyed the emission from a oak species (*Q. semecarpifolia*) which grows in Asia, is likely to be an ancestor of Mediterranean oaks, and is widespread in Himalayan forests (De Lillis et al. 1995).

## Materials and methods

Plants of *Q. pubescens*, *Q. robur*, *Q. ilex*, *Q. rotundifolia* and *Q. cerris*, 4–5 year old, were purchased at local nurseries. Three-year-old seedlings of the Asiatic species *Q. semecarpifolia* were grown from seeds collected during a scientific expedition in the Western Nepal forests (De Lillis et al. 1995). All plants were potted in 20-l pots, and maintained under optimal water and nutrient conditions while growing at a CNR experimental field in Roma. Plants of *Q. rotundifolia* and *Q. cerris* were also grown at the Joint Research Centre of the European Community in Ispra (Varese, Italy) in a greenhouse to avoid frost during winter. The emissions reported were measured at 30°C and 1000 µmol photons $m^{-2}$ $s^{-1}$ using single-leaf or branch cuvettes under synthetic air with atmospheric gas concentrations (80% $N_2$, 20% $O_2$, 350 ppm $CO_2$) but absolutely free of hydrocarbons. Isoprenoid analysis was carried out by gas chromatography and mass spectrometry. The gas-exchange apparatus and the system to collect and detect isoprenoid emission in Roma has been described in detail by Loreto et al. (1996). In Ispra emissions were collected by pumping the air exiting the cuvette at a rate of 100 ml $min^{-1}$ through a Tenax TA trap placed in a ice bath to avoid escape of volatile compounds. After collecting 1 l of air the trap was removed, desorbed through a Thermal Desorption Cold Trap Insector Purge and Trap Insector (TCT/PTI) desorption unit (CP 4001, Chrompack) and terpene content was analyzed by gas chromatography using a fused silica capillary column (CP-Sil 8 CB, Chrompack) and a flame ionization detector. All values reported from other studies (Hansen and Seufert 1996; Steinbrecher et al. 1997) were normalized to the emission at 30°C and 1000 µmol photons $m^{-2}$ $s^{-1}$ using the algorithm outlined by Ciccioli et al. (1997).

## Results and discussion

We found that *Q. pubescens* and *Q. robur*, two species of the subgenus *Quercus*, emit only isoprene. *Q. ilex*, and *Q. coccifera*, two species of the subgenus *Sclerophyllodrys*, emit 14 different monoterpenes but do not emit isoprene. Finally, species of the subgenus *Cerris* (*Q. cerris* and *Q. suber*), do not emit isoprenoids in detectable amounts. These data, as well as data reported in the literature about isoprenoid emission by other Mediterranean *Quercus* spp., suggest an identity between the chemo-taxonomy of oaks based on emission type and the morpho-taxonomy reported by the European flora (Schwarz 1964; Table 1).

The morpho-anatomical classification is mainly based on leaf sclerophylly, evergreeness, and fruit characteristics. However, such a classification is often genetically uncertain since anatomical characters are extremely dependent on environmental conditions, particularly in oak species that have extended natural ranges. In order to prove that morpho-anatomical differences correctly categorize plant taxa, a comparison with true genetic differences should be carried out.

While there have been several studies on the genetic differences of deciduous oaks, very little is known about the genetics of evergreen oaks. In one study, the genetic difference was estimated from allele frequencies at loci encoding for different enzymes (Samuel et al. 1995). A low genetic distance (*D*) was found between oaks of the subgenus *Quercus* ($D < 0.10$ for all species), while *Q. cerris* ($D > 0.62$) was genetically distant (Fig. 1). Another study based on chloroplast DNA restriction sites indicated, consistently with previous morpho-taxonomic studies, that the Euro-Asiatic evergreen oaks of the subgenus *Ilex* constitute a different clade from the other Euro-Asiatic subgenus *Cerris* (Manos 1997). On the other hand, sequences of nuclear ribosomal DNA indicated only a large clade, *Ilex* + *Cerris* (Manos 1997). Finally, the same taxonomic classification based on morpho-anatomy was also found by electrophoretic analysis of seed storage protein dissimilarities (Bellarosa et al. 1994; Fig. 1). Thus, it seems generally confirmed

**Table 1** Emission of isoprenoids from leaves of Mediterranean oaks as related to the taxonomic classification of *Quercus* spp. based on morpho-anatomy (Schwarz 1964). Emissions from *Q. petrea*, *Q. frainetto* and *Q. suber* are reported by Steinbrecher et al. (1997). Emission from *Q. coccifera* is reported by Hansen and Seufert (1996). The emission of Mediterranean oaks is also compared with the emission from two evergreen species considered closely related to *Q. ilex* on the basis of morpho-anatomy but native to North-America (*Q. agrifolia*, Simon et al. 1997) and the Himalaya range (*Q. semecarpifolia*). Data reported by other authors are shown in italics

| Species | Subgenus | Emission type and amount (nmol $m^{-2}$ $s^{-1}$) | |
|---|---|---|---|
| Mediterranean oaks | | | |
| *Q. pubescens* | Quercus | Isoprene | (26.1) |
| *Q. robur* | ” | ” | (7.8) |
| *Q. petrea* | ” | ” | (*4.3*) |
| *Q. frainetto* | ” | ” | (*30.7*) |
| *Q. ilex* | Sclerophyllodrys | Monoterpenes | (9.5) |
| *Q. coccifera* | ” | ” | (*8.8*) |
| *Q. rotundifolia* | ” | ” | (7.1) |
| *Q. cerris* | Cerris | Undetectable | (< 0.001) |
| *Q. suber* | ” | ” | (*< 0.001*) |
| Non-Mediterranean oaks with morpho-anatomical affinities to *Q. ilex* | | | |
| *Q. agrifolia* (North America) | | Isoprene | (*9.7*) |
| *Q. semecarpifolia* (Asia) | | Isoprene | (35.0) |

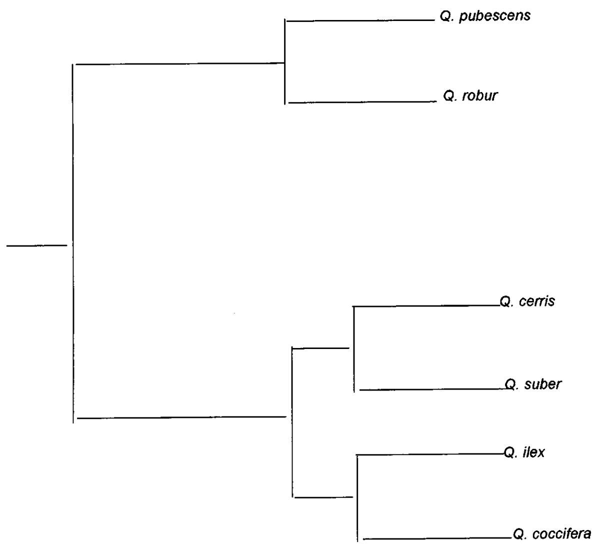


**Fig. 1** Simplified dendrogram showing the genetic relationships between the main Mediterranean oak species tested for isoprenoid emission. The dendrogram combines results obtained by studying genetic distances between *Quercus pubescens, Q. robur* and *Q. cerris*, as estimated from the frequency of alleles at the loci encoding for different enzymes (Samuel et al. 1995), and dissimilarities of seed storage proteins of all species, as estimated from electrophoretic analysis (Bellarosa et al. 1994)

that the different patterns of isoprenoid emission correspond to a true genetic difference among subgenera of the genus *Quercus*.

Interestingly, all North American oaks that have been screened for isoprenoids emit only isoprene in relevant amounts (Lamb et al. 1987), while no species that emit monoterpenes or lack isoprenoid emission have ever been found. This is true for both deciduous and evergreen oak species irrespective of the geographic distribution (Benjamin et al. 1996). As a specific case we cite *Q. agrifolia*, a live oak widespread in coastal California, which is closely related to the monoterpene-emitting *Q. ilex* on the basis of morpho-anatomy, but which emits only isoprene (Simon et al. 1997).

Nothing is known about the emissions of Asiatic oaks. We measured emissions from *Q. semecarpifolia*, an evergreen species which grows at high elevation in the forests of western Nepal. Despite the different habitat, *Q. semecarpifolia* anatomical characteristics are very similar to those of Mediterranean oaks. Krüssmann (1978) assigned *Q. semecarpifolia* to a section *Suber* including *Q. suber* and *Q. coccifera*. More recently, *Q. semecarpifolia* has been considered taxonomically related to *Q. ilex* (Ohsawa et al. 1986; De Lillis et al. 1995). Oaks that have colonized the Mediterranean region may originate from this Asiatic species (Ohsawa et al. 1986). The idea that Mediterranean oaks derive from Asiatic ancestors has been also given support from molecular studies on the Euro-Asiatic subgenera *Cerris* and *Ilex* (Manos 1997). For this reason, we expected to detect monoterpene emission (as for *Q. ilex*) or no emission (as for *Q. cerris* and *Q. suber*). However, like the North American relative of *Q. ilex*, *Q. semecarpifolia* was found to emit only isoprene, while no monoterpenes were detected (Table 1).

Isoprenoid synthesis and emission may be an adaptive trait since they are reported to increase tolerance to heat stress (Sharkey and Singsaas 1995; Loreto et al. 1998). If adaptation drove the formation of both morpho-anatomical characteristics and monoterpene synthesis, the identity between emission type and the current taxonomy of European oaks is partially explained. Monoterpene emitters have more sclerophyllous leaves than isoprene emitters, are evergreen, and are distributed in Mediterranean areas where temperature extremes and aridity are frequent. The emission of monoterpenes is generally lower than that of isoprene, but monoterpenes have higher molecular weight and are less volatile than isoprene. Consequently, the content of monoterpenes in the leaves is higher than that of isoprene. If all isoprenoids stabilize membranes against environmental constraints it is reasonable for species more exposed to these stresses to evolve the capability to form less volatile compounds which are more permanently involved in membrane physiology. However, the behaviour of non-emitters of the subgenus *Cerris* is not explained by considering isoprenoid formation as an adaptive trait, since they generally share morphological characteristics and geographical distribution with monoterpene emitters.

On the other hand, it is surprising that the great majority of oaks have evolved the capacity to produce isoprene despite the geographical distances and, in particular, the different habitats. More specifically, the fact that isoprene emission was found in oaks growing in the Himalaya range, on the west coast of United States, and in the Mediterranean region suggests that if the trait is adaptive it has evolved independently several times, perhaps to offer protection against a wide range of constraints which include but are not limited to high temperature.

We put forward the hypothesis that isoprenoid emission is not an adaptive character. Isoprene emission could be an ancestral genetic character in oaks, but it may have been either lost or modified by more recent clades such as those originating the Mediterranean oaks. This hypothesis is supported by the finding that an Asiatic oak, from which Mediterranean oaks are believed to have originated, emit isoprene. *Q. semecarpifolia* may be considered as a closely related outgroup with respect to Mediterranean oaks and as a possible origin for dendrograms based on chemo-taxonomy. If the emission of isoprenoids is not an adaptive character, then the taxonomy of European oaks, based on morpho-anatomy and on enzyme electrophoretic analysis, is fully validated by this independent trait. Were this the case, isoprenoid emission could serve as a chemo-taxonomical marker and could be used to reconstruct the phylogeny of oaks in association with molecular markers. This possibility deserves to be better tested. For instance, it would indicate that the link between *Q. semecarpifolia* and *Q. ilex* is not as strong as it was suggested by the similarity of the morpho-anatomical characteristics of the two species (De Lillis et al. 1995).

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