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# GEOGRAPHIC VARIATION IN TERPENE COMPOSITION OF *PINUS NIGRA* ARN.

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## ABSTRACT

The geographical variation in terpene composition of *Pinus nigra* was studied from 109 samples representing 72 natural and 11 planted populations. Relative amounts of 7 terpenes (camphene, ß-pinene, myrcene, Δ3-carene + terpinolene, ß-phellandrene and caryophyllene) were used for discrimination of populations. Five geographical groups could be distinguished: Spanish, French continental, Corsican, Calabrian and Eastern (comprising Austria, Balkan, Turkey, and Crimea). Evidence from our study of terpenes and from other data on anatomical, morphological and growth characters, and on terpenes, flavonoids and allozyme frequencies, support the use of the classification system of WHEELER *et al.* (1976).

**Keywords:** *Pinus nigra*, terpenes, taxonomy

## INTRODUCTION

European black pine has been considered as a single species only since 1957 when Röhrig recommended the retention of the name "*Pinus nigra*", given by Arnold in 1785. This species covers a discontinuous area of 2,300,000 ha, its range extends up to the northern Mediterranean coast, through 13 countries (Spain, France, Italy, Austria, Yugoslavia, Bulgaria, Albania, Romania, Greece, Turkey, Crimea, and isolated occurrence in Morocco and Algeria). *Pinus nigra* is highly polymorphic. There have been many attempts of classification, and more than a hundred different Latin names have been given to populations of the species, according to WRIGHT and BULL (1962). In 1974, VIDAKOVIĆ considered that no general consensus could be found about the taxonomy of the species. The confusion still exists since various classifications have been used in recent publications such as BONNET-MASIMBERT and BIKAI-BIKAI (1978), NIKOLIĆ and TUCIĆ (1983) and FARJON (1984).

We have investigated the geographical variation of *Pinus nigra* throughout its natural range by analyzing the terpene composition of cortical oleoresin (monoterpenes and sesquiterpenes) by gas-liquid chromatography. The relative amount of some mono- and sesquiterpenes has been found to show simple monogenic inheritance with a pair of alleles ('richness' and 'poorness' alleles) exhibiting variable dominance relationships. This simple genetic control seems to be rather general especially in the genus *Pinus*, e.g. in *Pinus pinaster* for Δ3-carene, limonene, myrcene, longifolene and caryophyllene (BARADAT *et al.* 1972, 1975, MARPEAU *et al.* 1975, MARPEAU-BÉZARD *et al.* 1983), in *Pinus taeda* for limonene, myrcene, ß-pinene, and ß-phellandrene (SQUILLACE *et al.* 1980), and in *Pinus sylvestris* for Δ3-carene, ß-pinene, ß-phellandrene, limonene and myrcene (YAZDANI *et al.* 1985). Although inheritance of terpenes was not studied in the case of *Pinus nigra*, we assumed that their synthesis was under similar genetic control. As such, they should constitute a reliable criterion for studies on population variation.

## MATERIAL AND METHODS

### Material

The study concerned 109 origins representing 72 natural and 11 planted populations of *Pinus nigra* (one population can be represented by more than 1 original sample, see table 1). Samples No 73, 74 and 75 were the result of controlled crosses, made with mixed pollen of five trees, of Corsican × Corsican, Calabrian × Calabrian and Corsican × Calabrian trees. Locations of ori-gins are given in Table 1 and Figure 1. Sampling was carried out in origin trials planted in 4 different locations in France: Amance (Meurthe et Moselle), Verdun (Meuse), Cendrieux and Cadouin (Dordogne); trees were between 5 and 13 years old when sampled. This age ensured a good expression of the genotypes for all the terpenes studied (BERNARD-DAGAN *et al.* 1972).

**Table 1 Geographical location of the native stands of *Pinus nigra* from which seed was collected for the provenance tests**

| No. | Abbreviation | Provenance * | Country | Latitude | Longitude |
|---|---|---|---|---|---|
| 1 | TIKJ | Tikjda | Algeria | 36°21 N | 3°55 E |
| 2 | CAZO | Cazorla | Spain | 37°50 N | 3°00 W |
| 3 | RO-MA | Rio Madera (2) | Spain | 37°50 N | 3°00 W |
| 4 | FRES | Fresneda | Spain | 39°50 N | 2°10 W |
| 5 | OLRE | Olmedadel Rey | Spain | 39°50 N | 2°10 W |
| 6 | PRIE | Priego | Spain | 40°30 N | 2°20 W |
| 7 | AINS | Ainsa | Spain | 42°30 N | 0°12 E |
| 8 | SGUL | Saint Guilhem (2) | Corsica | 43°41 N | 3°35 E |
| 9 | GAGN | Gagnères | Corsica | 44°07 N | 4°17 E |
| 10 | TART | Tartagine (5) | Corsica | 42°29 N | 8°57 E |
| 11 | VALD | Valdoniello (4) | Corsica | 42°18 N | 8°57 E |
| 12 | AITO | Aitone | Corsica | 42°17 N | 8°51 E |
| 13 | GHIS | Ghisoni | Corsica | 42°05 N | 9°12 E |
| 14 | PALN | Palneca | Corsica | 41°59 N | 9°12 E |
| 15 | MARM | Marmano | Corsica | 42°01 N | 8°70 E |
| 16 | VEZZ | Vezzani (2) | Corsica | 42°09 N | 9°15 E |
| 17 | SORB | Col de Sorba | Corsica | 42°08 N | 9°12 E |
| 18 | VIZZ | Vizzavona (3) | Corsica | 42°13 N | 8°67 E |
| 19 | ASPR | Aspromonte | Italy | 38°05 N | 16°00 E |
| 20 | CANT | Catanzaro | Italy | 38°54 N | 16°34 E |
| 21 | COZE | Cosenza (2) | Italy | 39°15 N | 16°17 E |
| 22 | TR-CO | Trenta Coste | Italy | 39°25 N | 16°35 E |
| 23 | MA-TA | Macchia del la Tav | Italy | 39°25 N | 16°35 E |
| 24 | GRAN | Grancia (2) | Italy | 39°25 N | 16°35 E |
| 25 | VI-BA | Villeta Barrea (2) | Italy | 41°47 N | 13°46 E |
| 26 | HOHE | Hohewand | Austria | 47°49 N | 16°00 E |
| 27 | DOBL | Doblhoff | Austria | ** | ** |
| 28 | WOLF | Wolfsohl | Austria | 47°43 N | 16°03 E |
| 29 | LEDE | Ledererkogel | Austria | 47°16 N | 16°53 E |
| 30 | MONO | Moldova Noua | Rumania | 45°41 N | 21°40 E |
| 31 | BANA | Banat | Rumania | 45°02 N | 20°40 E |
| 32 | DE-PE | Deliblatski Pešak | Yugoslavia | 44°48 N | 21°12 E |
| 33 | TRBO | Trbovle | Yugoslavia | 46°10 N | 15°03 E |
| 34 | BUGO | Bugojno (2) | Yugoslavia | 44°05 N | 17°25 E |
| 35 | TAZL | Tara Zlatibor | Yugoslavia | 43°40 N | 19°35 E |
| 36 | VGRA | Višegrad (3) | Yugoslavia | 43°90 N | 19°20 E |
| 37 | STUDII | Studenica | Yugoslavia | 43°30 N | 20°33 E |
| 38 | PLEV | Pljevlja | Yugoslavia | 43°22 N | 19°23 E |
| 39 | NIKS | Nikšić | Yugoslavia | 42°46 N | 18°56 E |
| 40 | BRAC | Brač | Yugoslavia | 43°20 N | 16°40 E |
| 41 | GR-AN | GradeskiAndek | Yugoslavia | 41°27 N | 20°38 E |
| 42 | PO-LV | Popova-Livada | Yugoslavia | 41°10 N | 21°55 E |
| 43 | TCHE | Tchernikov Borum | Bulgaria | ** | ** |
| 44 | KUST | Kustendil (3) | Bulgaria | 42°16 N | 22°40 E |
| 45 | RILA | Rila | Bulgaria | 42°08 N | 23°33 E |
| 46 | TZAR | Tzavaritza | Greece | ** | ** |
| 47 | THAS | Thassos (2) | Greece | 40°40 N | 24°40 E |
| 48 | PARA | Paramestion | Greece | 40°04 N | 24°04 E |
| 49 | KOSA | Kosani | Greece | 40°18 N | 21°47 E |
| 50 | GREV | Grevena | Greece | 40°05 N | 21°25 E |
| 51 | METS | Metsovon | Greece | 39°46 N | 21°11 E |
| 52 | MILE | Mile | Greece | 39°46 N | 21°11 E |
| 53 | ALON | Alonia | Greece | ** | ** |
| 54 | BASP | Barkazorema Spilaki | Greece | ** | ** |
| 55 | KA-ZE | Kapidag Zemliker | Turkey | 39°35 N | 27°00 E |

**Table 1 (continued)**

| No. | Abbreviation | Provenance * | Country | Latitude | Longitude |
|---|---|---|---|---|---|
| 56 | KARA | Karabelen | Turkey | 40°06 N | 29°02 E |
| 57 | ALAC | Alaçam (2) | Turkey | 39°35 N | 28°35 E |
| 58 | SIMA | Simav | Turkey | 39°00 N | 29°00 E |
| 59 | MUGL | Mugla | Turkey | 37°20 N | 28°24 E |
| 60 | YILA | Yilanli | Turkey | 37°00 N | 28°00 E |
| 61 | KAYM | Kaymakçi | Turkey | 36°52 N | 29°22 E |
| 62 | EGRD I | Egridir | Turkey | 37°40 N | 31°10 E |
| 63 | AFYO | Afyontarlas | Turkey | 38°07 N | 30°06 E |
| 64 | DEMR | Demirören | Turkey | 39°53 N | 29°46 E |
| 65 | CATA | Catacik | Turkey | 39°44 N | 31°04 E |
| 66 | DEGR | Degirmendere | Turkey | 39°57 N | 31°05 E |
| 67 | KURB | Kurbacik | Turkey | 40°30 N | 31°42 E |
| 68 | GEBE | Gebeler | Turkey | 40°30 N | 32°40 E |
| 69 | KOPR | Köprücek | Turkey | 37°00 N | 33°00 E |
| 70 | CANG | Cangal | Turkey | 42°00 N | 34°00 E |
| 71 | AYAN I | Ayancik I | Turkey | 41°43 N | 34°31 E |
| 72 | CRIM | Crimea Yalta (5) | Ukraine | 44°40 N | 34°20 E |
| **Planted populations** | | | | | |
| 73 | LRCO*LRCO | Les Barres (VALD*VALD) | | | |
| 74 | LRCA*LRCA | Les Barres (COZE*COZE) | | | |
| 75 | LRCO*LRCA | Les Barres (CCOZE*VALD) | | | |
| 76 | LBAR IIG | Les Barres | (France) | 47°50 N | 2°45 E |
| 77 | KOEK II | Koekelare II | Belgium | 51°04 N | 2°58 E |
| 78 | CAUS | Les Causses | France | 44°05 N | 3°07 E |
| 79 | EMBR | Embrunais | France | 44°34 N | 6°28 E |
| 80 | LARA | Laragnais | France | 49°28 N | 3°84 E |
| 81 | MEND | Mende | France | 44°30 N | 3°30 E |
| 82 | VL-HA | Ville Haute | France | 43°58 N | 6°52 E |
| 83 | LBPA | Les Barres | France | 47°50 N | 2°45 E |

* in brackets: number of samples (if more than one)
** unknown location

## Constitution of a mean sample

For each origin, at least 30 trees from different plots were sampled. From a top lateral branch of each tree, the shoot of the previous year's whorl was collected. Such shoots are assumed to be in the same physiological state. Weak trees, or trees attacked by insects were not sampled because terpene composition might be locally modified. Every shoot was stripped of a ring of cortical tissue and the 30 samples from one origin were mixed together to form the mean sample (fresh weight between 10 and 15 g). Individual terpene composition were not taken into account since individual terpene percentages are often not normally distributed, causing troubles for the tests realized after ANOVA.

## Terpene composition analysis

Tissues were minced and macerated for three hours in 30 to 50 ml of pentane (99%). The liquid phase was then purified on silica gel and analyzed by gas chromatography as described in MARPEAU *et al.* (1989).

## Choice of terpenes

The proportions of 8 monoterpenes (α-pinene, camphene, ß-pinene, myrcene, Δ-3-carene, limonene, ß-phellandrene and terpinolene) and one sesquiterpene (caryophyllene) were plotted on the chromatogram. Peaks were identified by comparison with reference

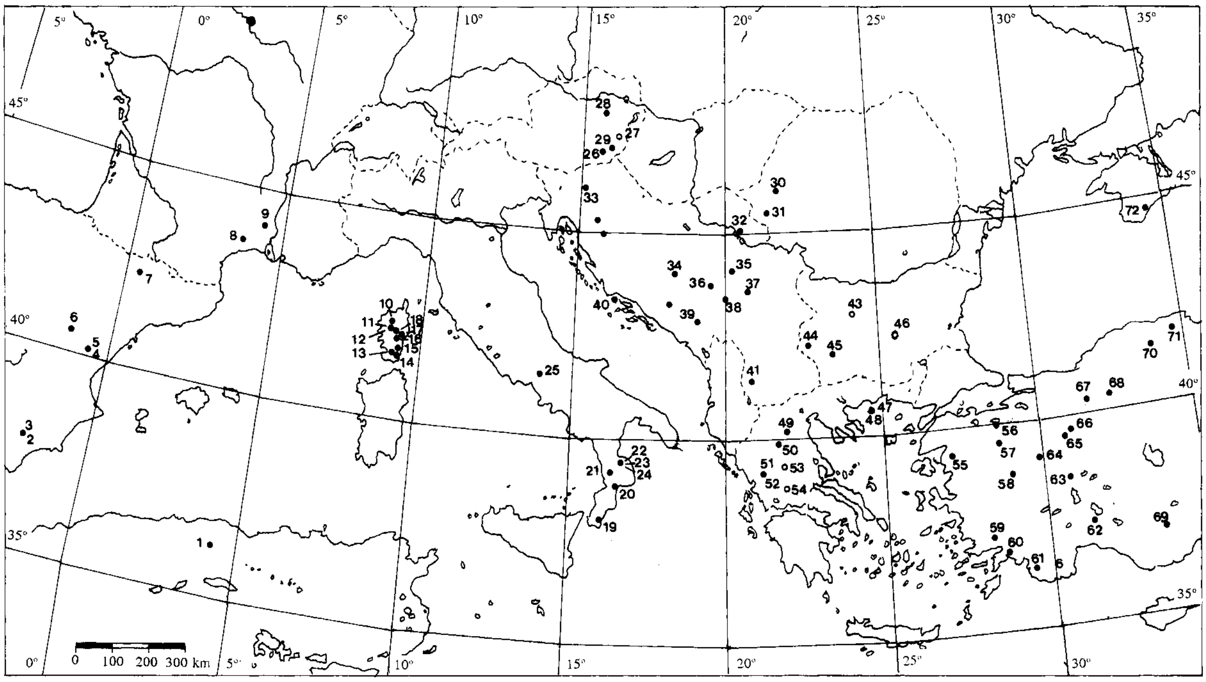


**Figure 1** Geographical location of the 72 native stands from which seed was collected for provenance tests

chromatograms run under the same conditions with known terpenes. The relative amount of each monoterpene was expressed as a percentage of the total monoterpenes and the amount of caryophyllene as a percentage of total terpenes. As the proportion of the ninth terpenes entirely determined by the 8 others, we decided to set apart one of the 9 terpenes. α-pinene is present in great quantity (a mean of 67% in the present analysis) in the oleoresin of conifers in general and pines in particular (see, for instance, VON RUDLOFF (1975), MIROV (1961)). Therefore it is the most affected by autocorrelation and its relative amount is mainly the conseuence of variation for other terpenes. These correlations between terpenes are partially due to their expression in percentage values. α-pinene has also often been found to exhibit low variation between populations for different species (VON RUDLOFF 1975, YAZDANI *et al.* 1985). For these reasons, the relative amount of α-pinene was not used in this study. The amount of limonene was also not taken into account, since its stable genotypic expression seems to be reached only at the age 8 to 10 in *Pinus piaster* Ait. (BARADAT *et al.* 1972); the situation tends to be the same for *Pinus nigra* (GERBER, unpublished data).

Since Δ-3-carene and terpinolene are under the same genetic control (ZAVARIN 1970, BARADAT & MARPEAU-BÉZARD 1988), the relative amounts of the two terpenes were added to form a single character.

### Statistical analysis

Individual percentages were transformed into $\sqrt{\text{arcsin}}$ (proportion) to normalize distribution of errors and to avoid a relationship between the error variance and the proportion of each terpene in the mean sample.

Each of the 109 mean samples was therefore represented by a set of 6 characters: relative amounts of camphene, ß-pinene, myrcene, Δ3-carene + terpinolene, ß-phellandrene and caryophyllene. A principal component analysis was performed, based on the correlation matrix computed with the 98 samples corresponding to the natural origins. The samples from planted populations were not included in the calculation of the axes but were considered as supplementary data.

## RESULTS

### Natural populations

Table 2 gives the general results of the principal component analysis for the 6 traits studied. The first 3 axes of the analysis, which represented 80 % of the variance, were retained. With the exception of 4 origins[1], the stands cluster into distinct geographical groups. We considered the relative positions of the groups, on

1)
- one from Spain (No.3, Rio Madera) and one from Corsica (No.12, Aïtone) situated among origins of the eastern part of the natural area,
- one from Villeta Barrea (No.25, Italy) situated among Corsican populations,
- one from Turkey (No.58, Simav) rather isolated.

**Table 2 General results of the principal component analysis: eigenvalues in % of the total variance and correlation with initial traits for the first three axes**

| | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|
| Eigenvalues (%) | 34 | 29 | 17 |
| Cumulative eigenvalues (%) | 34 | 63 | 80 |
| Initial traits | Correlations | | |
| camphene | 0.67 | –0.16 | –0.60 |
| ß-pinene | 0.25 | 0.64 | 0.55 |
| myrcene | –0.87 | 0.17 | –0.17 |
| Δ3-carene + terpinolene | –0.70 | –0.21 | –0.30 |
| ß-phellandrene | –0.33 | –0.72 | 0.46 |
| caryophyllene | –0.36 | 0.83 | –0.18 |

**Table 3 Mean amounts of terpenes in the groups from the principal components analysis**

| Group | camphene | ß–pinene | myrcene | Δ3–carene | ß–phellandrene | caryophyllene |
|---|---|---|---|---|---|---|
| **Major groups** | | | | | | |
| Spain (7) [1] | 1.16 | 13.02 | 16.78 | 2.31 | 0.89 | 7.97 |
| Corsica (19) | 1.63 | 5.38 | 3.98 | 1.12 | 7.72 | 1.87 |
| East (60) | 1.74 | 15.28 | 2.47 | 0.40 | 2.04 | 4.09 |
| **Middle groups** | | | | | | |
| Calabria (8) | 2.04 | 4.46 | 1.44 | 0.30 | 0.63 | 1.53 |
| France (3) | 1.77 | 5.47 | 6.95 | 0.32 | 1.50 | 9.23 |
| **Minor groups** | | | | | | |
| East (38) [2] | 1.70 | 10.57 | 2.37 | 0.37 | 1.16 | 4.36 |
| Turkey (18) | 1.63 | 22.69 | 2.49 | 0.49 | 1.46 | 4.60 |
| Austria (4) | 1.88 | 12.58 | 2.55 | 0.34 | 3.49 | 3.31 |
| Algeria (1) | 1.73 | 16.97 | 5.63 | 0.35 | 0.99 | 5.41 |

[1] in brackets: number of samples in the group
[2] without Turkey and Austria

Figure 2 and 3, combined with the correlations between axis and initial characters (Table 2). The mean amounts for the 6 terpenes calculated for each group are given in Table 3. The main features of the terpene composition discriminating these groups from each other can be derived from these data (table 4).

Three "major" groups are clearly distinct: a Spanish group, a Corsican group and an Eastern group (Austrian, Rumanian, Yugoslavian, Bulgarian, Greek, Turkish and Crimean origins). They are especially differentiated from each other on the first plane (axis 1 and 2). Their main distinctive features are summarized on Table 4.

Two additional "middle" groups can be distinguished, relative to the major groups. A Calabrian group between the Corsican and the Eastern group, in the first plane and a French continental group, comprising only three samples but clearly clustered between Spanish and Eastern groups, though closer to the latter group. ARBEZ *et al.* (1974), studying 3 mean samples of Calabrian and Corsican origins established the same results for the differences of terpene composition between these two groups. French continental origins are radically different from Spanish origins in their terpene composition on the basis of their values on the first coordinate (see Table 4). These differences for myrcene and camphene were also found by ARBEZ *et al.* (1974) between one French and two Spanish samples. Our single sample from Algeria (No.1), is part of the Eastern group.

"Minor" groups could be detected within the Eastern group. Austrian origins are represented by only four samples but tend to have lower values on the first coordinate than the other members of the Eastern group (i.e. higher ß-pinene, Δ3-carene + terpinolene and lower ß-phellandrene, see Table 3), and tend to be closer to Calabrian samples.

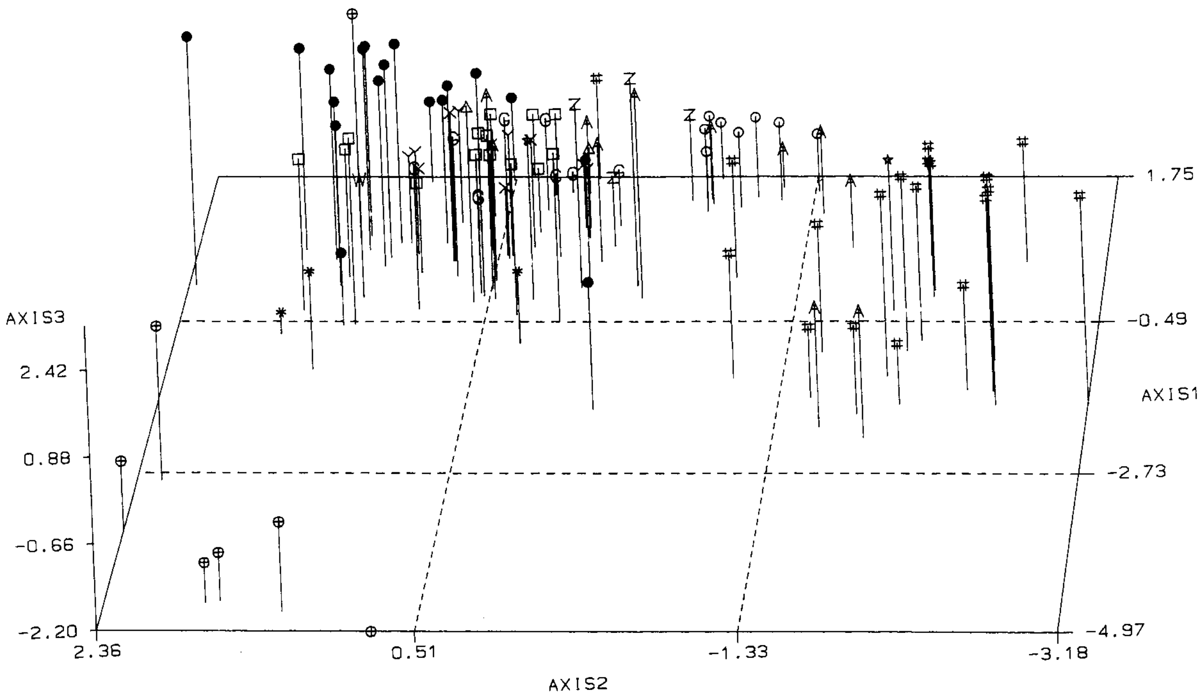


**Figure 2** The first three axes of the principal component analysis: samples identified by their origin: WWW – Algeria; ooo – Calabria, *** – France, ΔΔΔ – Rumania, ZZZ – Austria, ### – Corsica, GGG – Greece, ⊕⊕⊕ – Spain, xxx – Bulgaria, YYY – Crimea, ☆☆☆ – Italy, ●●● – Turkey, □□□ – Yugoslavia, AAA – artificial

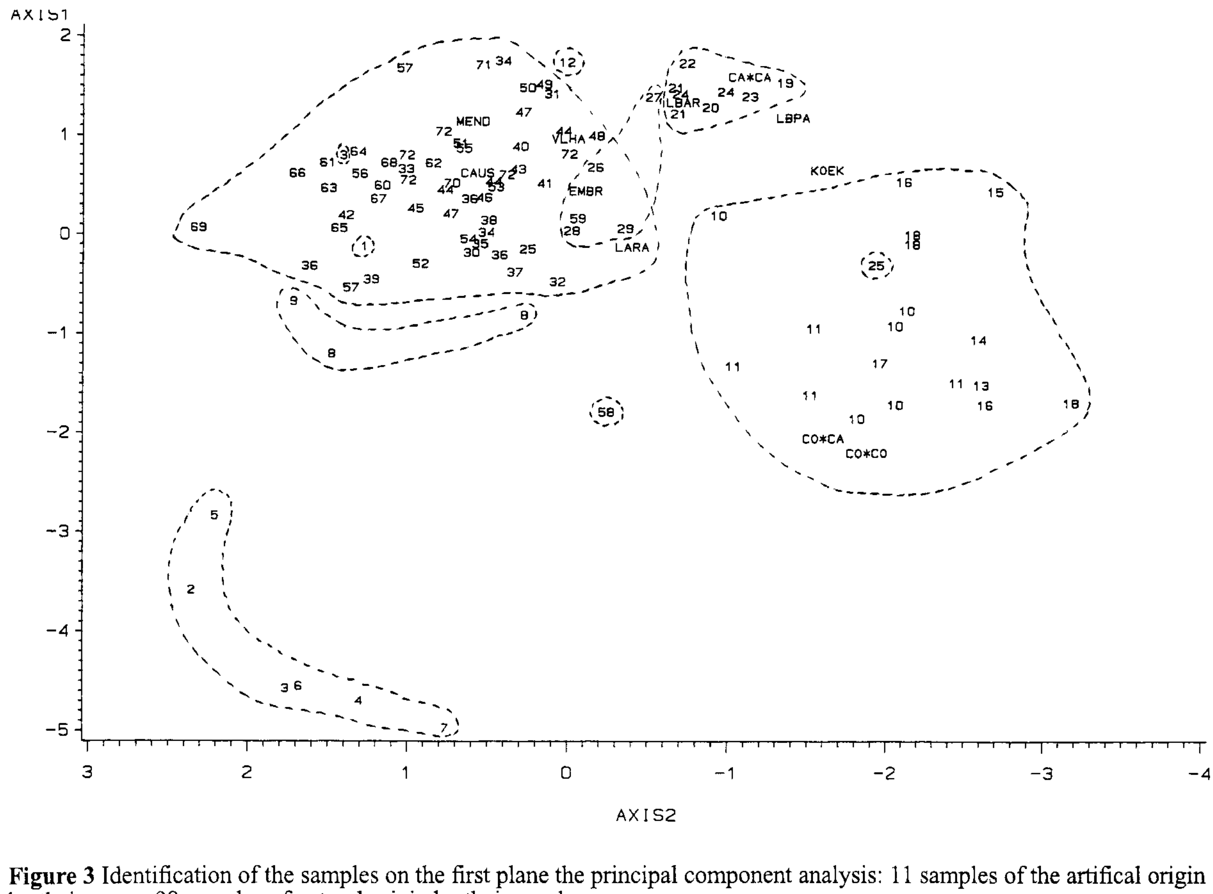


**Figure 3** Identification of the samples on the first plane the principal component analysis: 11 samples of the artifical origin by their name, 98 samples of natural origin by their number

Some Turkish samples show a tendency to have higher values on the third axis and thus higher ß-pinene than the Yugoslavian samples (clearly seen on table 3). Yugoslavian stands clustered together with Greek, Crimean, Bulgarian and Rumanian origins. A principal component analysis carried out only with these Eastern origins did not reveal any clearer geographical pattern. A slight differentiation can be noticed among these origins, but they are nevertheless close to each other in terpene composition.

### Planted populations

Terpene composition of the planted populations is given in table 5. These samples were processed as supplementary data (figures 2 and 3):

- French planted stands (Nos. 78 to 82), known to be of Austrian origin (a variety which was largely used for reforestation in France) are actually part of the Eastern group;
- The progeny of a stand called "les Barres *pallasiana*" (No. 83) originating reputedly from the extreme East of the natural area, is more likely to be of Calabrian or Corsican origin according to its terpene composition, and also from its morphology (ROMAN-AMAT 1984). This result may be due to the close proximity of Calabrian and Corsican stands in the area from which the progeny comes, which may consequently have been the parents by natural pollination;
- Koeklare (No. 77), a Belgian origin, known to have been introduced during the invasion of the country by Napoleon, but supposed to be intermediate between Calabrian and Corsican pines (NANSON 1972), could indeed be so according to its intermediate position between these two groups;
- The population resulting from a controlled cross of Corsican (No. 73) and Calabrian (No. 74) pines are part of the parental group. The mean hybrid between Corsican and Calabrian pines clusters with the Corsican group.

## DISCUSSION

Terpenes are known to be involved in plant-insect relationships (*Pinus nigra*: CHARLES *et al.* 1982) but the possible importance of selection pressure on genes which control their biosynthesis has not been quantified. Their neutrality as markers is thus often disputed. In contrast, allozymes or DNA markers are considered to be neutral markers, providing weak geographical discrimination, compared to terpenes. However, in several

**Table 4 Major features of the terpene composition discriminating the geographical groups on the principal component analysis**

| | | |
|---|---|---|
| (a) | **Spanish group:**<br>(axis 1) | higher myrcene<br>higher Δ3–carene + terpinolene<br>lower camphene |
| (b) | **Corsican group**<br>(axis 1) | lower caryophyllene<br>higher ß-phellandrene<br>lower ß–pinene |
| (c) | **Eastern group**<br>on axis 1<br>relative to Spanish group | lower myrcene<br>lower Δ3–carene + terpinolene<br>higher camphene |
| | on axis 2<br>(relative to Corsican group) | higher caryophyllene<br>lower ß–phellandrene<br>higher ß–pinene |
| (d) | **Calabrian group**<br>(relative to Corsican group) | lower myrcene and Δ3–carene + terpinolene<br>higher camphene<br>lower ß–phellandrene |
| (e) | **French continental group** | lower myrcene and Δ3–carene + terpinolene<br>higher camphene |

**Table 5 Mean amount of terpenes (in %) in samples from planted populations**

| Population | camphene | ß-pinene | myrcene | Δ3-carene+ terpinolene | ß-phellandrene | caryophyllene |
|---|---|---|---|---|---|---|
| EMBR | 1.87 | 11.93 | 2.09 | 0.52 | 2.66 | 3.80 |
| KOEK | 2.24 | 4.55 | 5.07 | 0.40 | 2.29 | 0.81 |
| LBAR | 2.13 | 7.12 | 2.05 | 0.32 | 0.93 | 1.63 |
| VLHA | 2.04 | 12.19 | 1.97 | 0.36 | 1.89 | 3.50 |
| LBPA | 2.21 | 6.43 | 2.44 | 0.40 | 2.08 | 1.02 |
| MEND | 2.11 | 13.93 | 2.53 | 0.49 | 0.10 | 2.93 |
| CO*CO | 1.65 | 3.68 | 11.93 | 0.89 | 10.96 | 2.71 |
| CA*CA | 2.31 | 3.09 | 1.47 | 0.35 | 0.59 | 1.47 |
| CO*CA | 1.66 | 3.51 | 12.33 | 0.65 | 9.48 | 2.95 |
| LARA | 1.34 | 16.09 | 2.35 | 0.44 | 5.20 | 2.74 |
| CAUS | 1.61 | 15.83 | 2.29 | 0.20 | 1.83 | 3.96 |

instances, the patterns of geographical variation displayed by terpenes and isozymes are the same, for example in a study of the native range of Douglas-fir (LI & ADAMS 1988). Using 20 enzyme loci, these authors found a large scale geographical discrimination in agreement with the one found with terpenes with only minor local differences. Terpene information seems therefore to be suitable for taxonomic purposes, comparable with data using other markers with high or complete genetic control.

In classical, descriptive taxonomy developed in 1750 by the Swedish botanist Linnaeus, a plant name should above all permit to identify the plant, to classify it and not to indicate its phylogeny (LAWRENCE 1955, cited in MIROV 1967). A taxonomist is obviously still asked to be able to identify plant material. This is usually possible with morphological characteristics but today, genetic markers can be added to these criteria. Actually, as WALTERS (1963) and CRAWFORD (1983) emphasize, "difference" or "resemblance" are relative terms, established by the context. No taxonomic character is representative of a whole genome so that a variety of approaches can be followed to clarify confused taxonomic situations, such as are found, for instance, in the genus *Pinus* (MIROV 1967) and *Salix* (WALTERS 1963). The ecological and geographical barriers between differentiating populations *of Salix* were destroyed during the glacial period. The present distinctions between taxa of recent origin, which have not diverged significantly, are difficult to define (CRONQUIST 1973). There is consequently no general agreement on the classification of the genus *Salix*. On a species and subspecies level, we are faced with the same difficulties for *Pinus nigra* Arn. We tried to interpret our results for *Pinus nigra*, in the context of those of other authors using different techniques.

The classification proposed in 1958 by FUKAREK for *Pinus nigra* and cited by DEBAZAC (1964), in his handbook on conifers, is generally used. As DEBAZAC (1964) wrote, it is essentially based on geographical distribution, and has the advantage of simplicity:

- subsp. *clusiana* in Northern Africa, Spain and France;
- •subsp. *laricio* in Corsica and Calabria;
- •subsp. *nigricans* in Austria, central Italy and Balkan;
- •subsp. *pallasiana* in Turkey and Crimea.

The distinction between the last two subspecies is not clear. DEBAZAC hesitates to place Greek population in the first or in the second subspecies. ARBEZ and MILLIER (1971) did not find any difference for four needle-characters between the subspecies. Small differences were found for the content of ß-pinene by ARBEZ *et al.* (1974), and for the frequency of one allele at the enzyme locus GOT by BONNET-MASIMBERT and BIKAY-BIKAY (1978). Our conclusions tend to discriminate the Turkish populations but cannot provide any clearer distinction between (and within) these subspecies. Moreover, results of earlier papers can be seen as underlying the absence of distinction between origins of this Eastern part of the natural area.

WRIGHT and BULL (1962), from an analysis of seed weight and 12 seedling characteristics, could only differentiate the Corsican populations from a large origin collection. In 1968, LEE measured 3 growth, 19 anatomical and 18 chemical traits in the same collection. He found trends of variation, but he grouped together Yugoslavian, Greek, and Turkish origins, setting apart Crimean and Austrian pines (with a single seedlot for each). WHEELER *et al.* (1976) measured height, mortality and winter injury on the same material, and added the last two origins, constituting one single group. An origin from Cyprus had different characteristics. This result is in agreement with the apparent distinction of a population of the same origin reported by BONNET-MASIMBERT and BIKAY-BIKAY (1978), based on allozyme frequencies at 4 loci. NIKOLIĆ and TUCIĆ (1983), studying allozyme variation at 4 loci on a large sample from this area, did not agree with the classification of the Eastern part of the natural range proposed by VIDAKOVIĆ in 1974. In fact, the different subspecies supposed to constitute these populations appear to be very close to each other, according to their new genetic distances. This lack of clear discrimination among populations distributed on a wide area may be explained by human intervention, acting over a long period on these forests, a point previously emphasized by DEBAZAC (1971).

## CONCLUSION

Calabrian and Corsican pines are clearly differentiated from the other origins and from each other on the basis of growth, anatomical and chemical characters (LEE 1968, WHEELER *et al.* 1976), number of cotyledons (RÖHRIG 1969), needle-characters (ARBEZ & MILLIER 1971), monoterpene composition (ARBEZ *et al.* 1974, FINESCHI & GROSSONI 1981), flavonoid compounds (LAURANSON 1989), and isozymes (BONNET-MASIMBERT & BIKAY-BIKAY 1978, FINESCHI 1983, NIKOLIĆ & TUCIĆ 1983, SCALTSOYIANNES *et al.* 1994). Our results lead to the same conclusions.

French and Spanish origins, included in the 1958 classification of FUKAREK into one single group are also clearly discriminated by different studies. LEE (1968), and subsequently WHEELER *et al.* (1976), clustered them in separate groups from their phenotypical features. Spanish populations appear to contain the most vigorous trees, on a 9 year old origin trial in New Zealand (WILCOX & MILLER 1974). From the discriminant analysis of BONNET-MASIMBERT and BIKAY-BIKAY (1978), one can see a clustering of Spanish origins clearly distinct from the french ones.

The relationships of Northern African origins within the species have not been ascertained. The information available is not consistent, since a Moroccan population appeared to be distinct from other populations (NIKOLIĆ & TUCIĆ 1983) whereas our Algerian sample can be included among Eastern origins. This populations supposed to be native but we could wonder whether it has been introduced during colonial times.

On the principal component analysis, the clustering of the planted populations included in our study with controls of natural origin is an illustration of the use of terpene composition to verify or specify the probable geographical origin of unknown stands. This technique is used in routine varietal identification of *Pinus pinaster* (BARADAT & MARPEAU-BÉZARD 1988).

In their conclusion, WHEELER *et al.* (1976), attempted to clarify the taxonomy of *Pinus nigra*, combining their results with those of RÖHRIG (1966) and WILCOX & MILLER (1974). We are inclined to join these authors in their classification proposal. Except for the Eastern part of the natural range, which does not correspond to a precise region, the classification suggested is close to an earlier idea of Röhrig who proposed, in 1957, to identify the different populations of *Pinus nigra* by their origin ("origin of" Calabria, Corsica,...). The classification is as follows:

*Pinus nigra* Arn.

- var. *pyreneica* (La Peyrouse) Godron for Spanish populations,
- • var. *salzmanii* (Dunal) Asch. and Graebner for French trees,
- • var. *poiretiana* (Ant.) Schneider for Corsican populations,
- • var. *calabrica* (Loud.) Schneider for Calabrian populations
- • var. *nigra* (Hoess) Asch. and Graebner for Central Italy, Austria,Yugoslavia, Albania, Greece, Romania, Bulgaria, Crimea, and Turkey.

Additional studies from all Eastern areas with genetic markers would lead to a better understanding of the geographical variation of this last and largest variety.

This study should help to define international policies for the of preservation of genetic diversity for this species.

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