GENE CONSERVATION IN PINE SPECIES

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ABSTRACT

Since a gene resource is a collection of biological material containing either specific or particularly variable genetic information, several cases can be found in European and Mediterranean pine species which meet these requirements and might be included in gene conservation programs. On the basis of their geographic distribution, European forest trees may show different patterns of variation among and within populations, as is inferred from isozyme markers; they may present several examples of populations carrying specific genetic information that can therefore be looked upon as gene resources. Other genetic markers (morphological, physiological and biochemical) are also useful to indicating existing differentiation among populations and provenances. In this connection, some significant cases are shown for the following species: *Pinus sylvestris* L., *Pinus leucedormis* Ant., *Pinus nigra* Arn., *Pinus cembra* L., *Pinus halepensis* Mill. For each of them the proposed form of preservation is mainly the dynamic gene conservation *in situ*, which makes it possible to preserve the genetic adaptability of populations in changing environments.

Keywords: *Pinus sylvestris*, *Pinus leucodermis*, *Pinus cembra*, *Pinus halepensis*, *Pinus nigra*, gene resource conservation, genetic differentiation, geographic range

INTRODUCTION

The concept of gene conservation requires a definition of the gene resources to be preserved. A gene resource can be defined as a collection of biological material containing either specific or particularly variable genetic information (ZIEHE *et al.* 1989). On the basis of this definition, we can find several examples of gene resources in European and Mediterranean pine species.

Depending on their geographic distribution, European forest trees may show different patterns of intra- and interpopulation variation, as is inferred from isozyme markers (MÜLLER-STARCK *et al.* 1992); populations that are derived from different glacial refugia, or belong to different subspecies or races, to different areas of discontinuous ranges, to species with extremely small ranges, or are locally isolated: all of them can carry specific genetic information and can therefore be looked upon as gene resources. This variation can be studied also through other markers, either morphological, physiological, or biochemical (terpenes and antigens), which sometimes indicate better than do isozymes existing differentiation among populations and provenances (PRUS-GŁOWACKI 1991).

The genus *Pinus* covers a very wide region in the Northern Hemisphere, mainly the north temperate zone (Fig. 1). It is one of the most important forest genera, and includes more than 100 species; of these, about 13 are found in Europe – but this number changes in accordance with different systematic classifications.

Among the European species, the following will be discussed in connection with some gene conservation problems: *Pinus sylvestris* L., *Pinus leucodermis* Ant., *Pinus nigra* Arn., *Pinus cembra* L., *Pinus halepensis* Mill.

Scots pine

Scots pine (*Pinus sylvestris* L.) occupies a larger area than any other pine species, and even any other species from the whole *Pinaceae* family (MIROV 1967; BORATYŃSKI 1991; Fig. 2).

Because of such a wide range, with very different environmental conditions, and because of the long history of this pine, a large intraspecific variation is expected to occur. Scots pine must have developed in East Asia then spreading towards Europe, where it has been present since the Tertiary Period. During the glacial and interglacial periods of the Pleistocene, its range underwent repeated modifications from which numerous new populations arose resulting in high differentiation and increased variability, so that it has been very difficult to create an intraspecific taxonomic system (MOLOTKOV & PATLAJ 1991). Several international provenance experiments on morphological (growth) traits have shown the existence, in the Baltic countries, of races endowed with great adaptability: these are important genetic resources, not only biologically but also economically; the above studies have

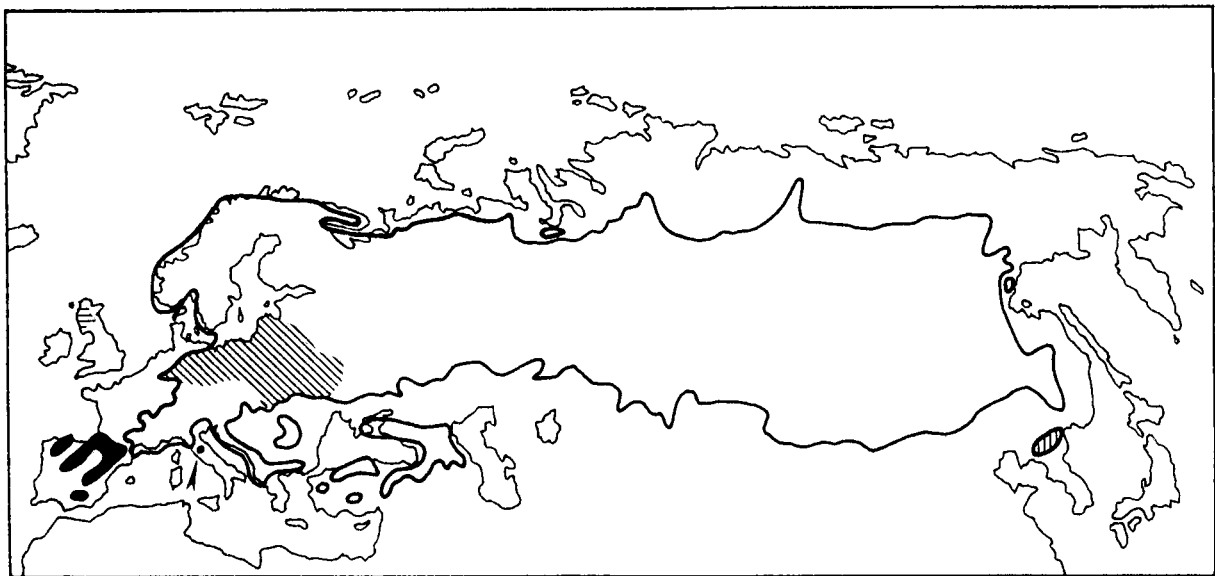**Figure 1** Natural range of the genus *Pinus* (after MIROV 1967)

Figure 2 Natural range of *Pinus sylvestris* L. (after BORATYŃSKI 1991). The hatched and black areas and the small arrow (close to Italy) point to the locations of some subspecies, races and populations which are important for gene resources conservation (see text)

also shown some marked differences among various provenances (GIERTYCH 1991).

OLEKSYN *et al.* (1992) proved the existence of photoperiodic ecotypes, which enable the European range of this species to be divided into three parts.

Geographic differentiation in the Scots pine natural range has been observed also by means of biochemical markers, namely antigens (immunochemical techniques), terpenes and isozymes (PRUS-GŁOWACKI 1991). Recently, molecular markers too have confirmed a phenomenon regularly observed in most conifers when using isozymes, that is to say only a small portion of total variation is caused by differentiation among
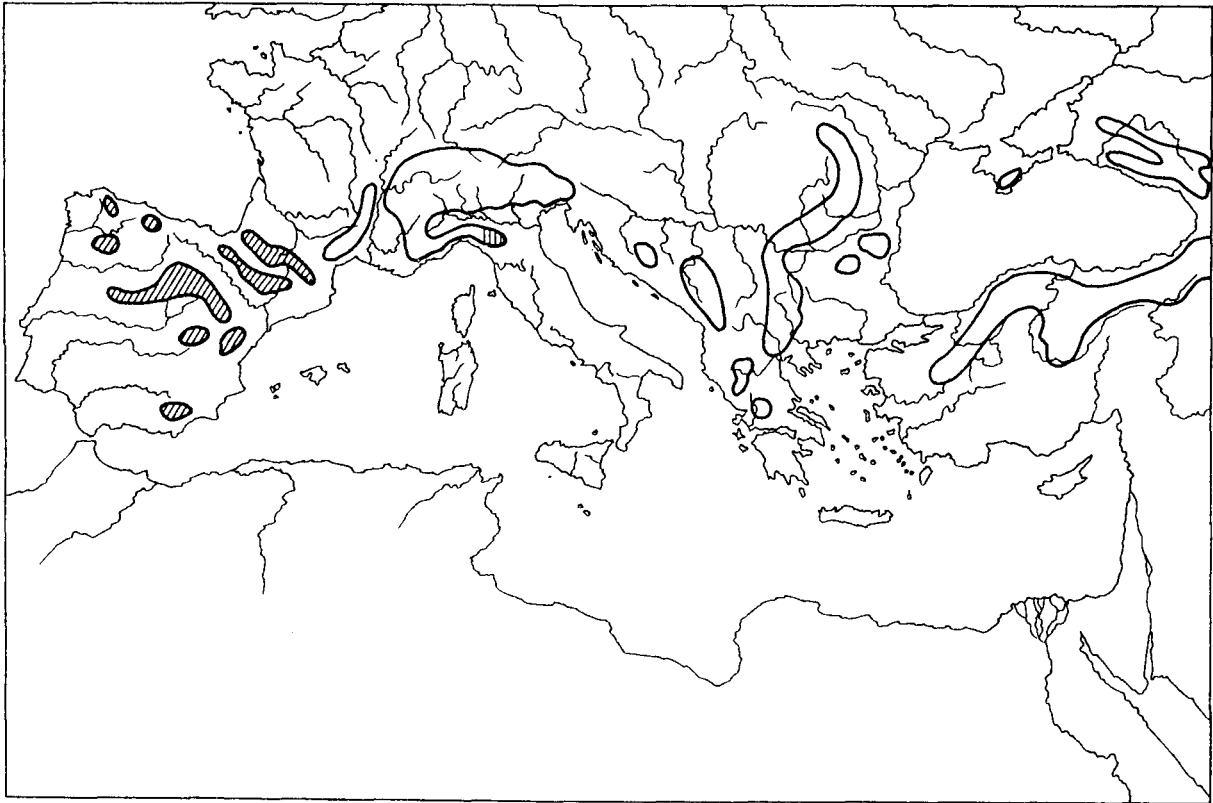
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Figure 3 Natural range of *Pinus sylvestris* L. in the Mediterranean region (after MIROV 1967). The hatched areas mark the locations of some populations which are important for gene resources conservation (see text)

populations. Some specific cases, however, are discussed in the following paragraphs.

WANG *et al.* (1991) studied enzyme differentiation among three Swedish populations (*P. sylvestris* var. *lapponica* (Fries) Hartman) and four Chinese populations (three belonging to var. *mongolica* Litvinov and one to var. *sylvestriformis* Takenouchi). In spite of the very long geographic distance between the populations of the varieties *lapponica* and *mongolica*, they do not seem to be very much differentiated, while the Chinese population belonging to the variety *sylvestriformis* is highly differentiated from the rest, although it grows in geographic proximity with the variety *mongolica*. The authors assumed it could be an interspecific hybrid between *P. sylvestris* and an eastern Asiatic pine, *Pinus densiflora* Sieb. et Zucc. Interspecific hybridization has played a significant role in the evolution of pine species in Asia, but a single population was not enough for a general statement to be made. Therefore, the authors concluded that the varieties *lapponica* and *mongolica* had derived from a common ancestral population after glaciers' withdrawal.

SZMIDT and WANG (1993) studied several populations for each taxonomic group by using isozymes and RFLP of paternally inherited chloroplast DNA: three populations were investigated for the var. *lapponica*,

four for the var. *armena* K. Koch, four for the var. *mongolica*, five for the var. *sylvestriformis* and six for *P. densiflora*. The study confirmed previous findings suggesting that introgression gave rise to the variety *sylvestriformis*, which thus becomes extremely valuable for gene resource conservation.

PRUS-GŁOWACKI and STEPHAN (1994) compared 7 Spanish and 16 North and East Europe populations by means of isozymes. The Spanish populations are strongly differentiated, especially the population representing the southern border of the species' natural range (Sierra Nevada; Fig. 3). The obtained results agree well with their being attributed respectively to the varieties *iberica* Svoboda, *pyrenaica* Svoboda and *nevadensis* Christ. They are scattered and isolated populations and, according to these results, must be looked upon as important genetic resources within this species (Fig. 3).

KINLOCH *et al.* (1986), using monoterpene and isozyme loci, found that some populations in Northwestern Scotland (Wester Ross) were differentiated from all others and from one another; they suggest an endemic origin of the Caledonian race of Scots pine from more than one glacial refugium, and since the studied Scottish populations are relict and show high values of expected heterozygosity, they require protection, especially the more differentiated ones.
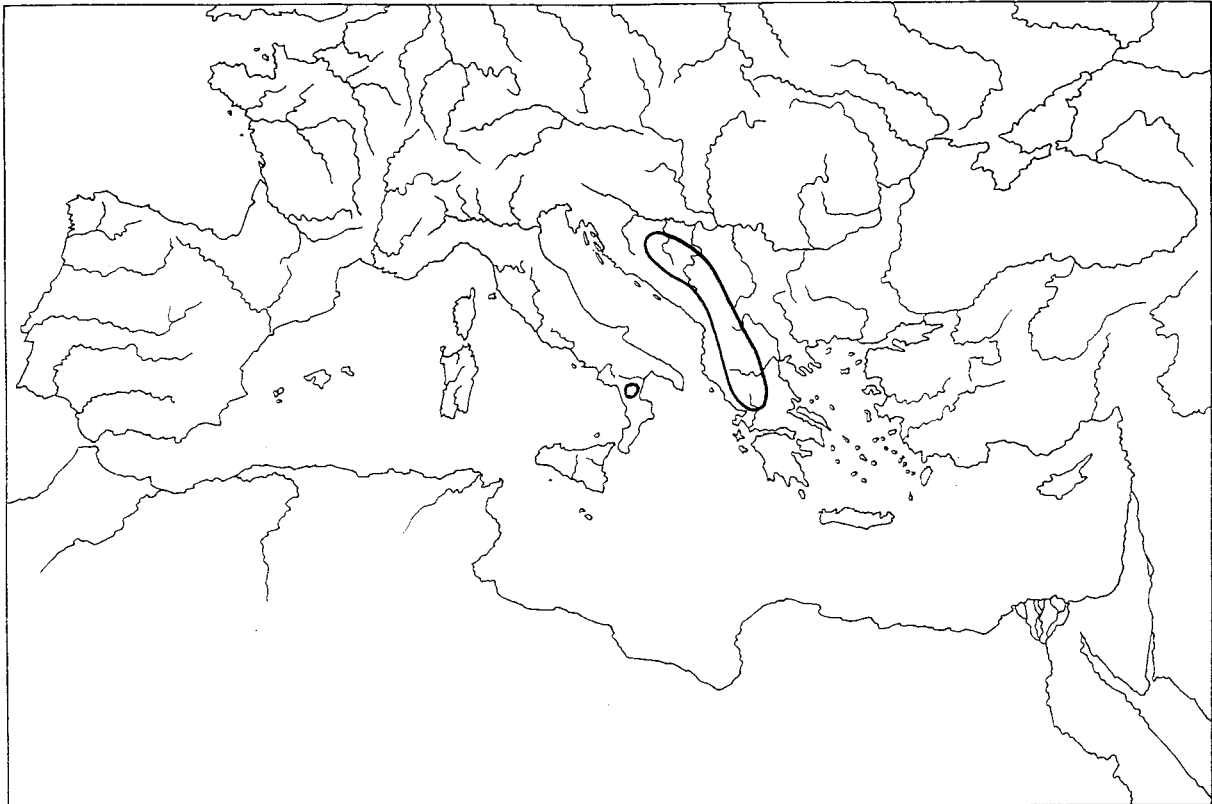
Figure 4 Natural range of *Pinus Heldreichii* Christ. and *Pinus leucodermis* Ant. (after MIROV 1967)

In Italy, Scots pine is present on the Alps and on the Northern Apennines (Fig. 3). The Apennine range consists of scattered and relict populations which bear witness to the climatic events of the inter- and postglacial periods (AGOSTINI 1972). Preliminary results obtained by PUGLISI and CIFARELLI (unpublished data), in a study on enzyme polymorphism of seven Alpine populations and one Apennine population, show that the latter is clearly differentiated from the Alpine populations, which are all alike; this clear differentiation reveals a fairly different evolutionary history, possibly due both to the isolation of the Apennine population and, if the postglacial recolonization of the Alps did not start from the refugia in the Northern Apennines and on the Tyrrhenian coasts near Viareggio (GIACOMINI 1958), to a different origin. The investigated relict population, whose intrapopulation genetic variation is as high as in the Alpine populations, is small and completely surrounded by cultivated stands. Since it is an official seed stand, it is protected by existing laws; however, due to its genetic value, more specific protection measures should be introduced, to include other Apennine relict populations as well.

Pinus leucodermis

In past times *Pinus leucodermis* Ant. used to be considered as a variety of *Pinus Heldreichii* Christ., and later
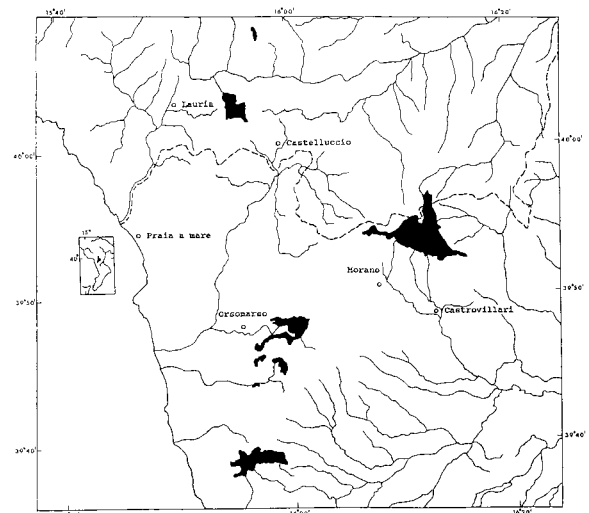

Figure 5 Natural range of *Pinus leucodermis* Ant. In Southern Italy (after AVOLIO 1984) (black areas after Avolio 1984)

as a distinct species (AVOLIO 1984). Its natural range is discontinuous and mainly in the Balkan region, but it also occurs as a volunteer species in a small area in Southern Italy, at the border between Basilicata and Calabria (Fig. 4). Here, only some minor and isolated relict populations survive, though in the past the Italian range was probably much wider (AVOLIO 1984; Fig. 5).

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BOSCHERINI *et al.* (1994) studied five Italian and two Greek populations (of which one is classified by some authors as *P. Heldreichii*): allozyme variation within and among populations is rather low, nevertheless the sampled Greek populations tend to differentiate from the Italian ones; RFLP analysis of chloroplast DNA, which is very useful to taxonomic studies on pine species, showed no differences between the sampled populations, so that they may belong to the same biological species, in accordance with Mirov's opinion (1967) who regarded *P. leucodermis* and *P. Heldreichii* as one and the same species.

Generally, species with very small ranges exhibit a relatively high differentiation among populations, though this does not apply to *P. leucodermis* (MÜLLER-STARCK *et al.* 1992). Possibly, its relatively low variation results from past genetic "bottlenecks" of local populations (BOSCHERINI *et al.*, 1994). Because of its small and discontinuous range, both in Italy and in the Balkans, *P. leucodermis* is an endangered species needing protective measures. The most important Italian population (Mt. Pollino) is in fact included in the National Park of Calabria.

Black pine

Pinus nigra Arn. is a collective species with a discon-

tinuous natural distribution. Problems connected with its geographic range (Fig. 6) and taxonomy have long been debated, both with respect to classification of various populations – often located a long distance away from one another – and in the light of the taxonomic significance of the different suggested entities (species, subspecies, varieties, biological and geographical races).

Geographic variation among provenances, studied on the basis of morphological and anatomical traits (GELLINI 1968; ARBEZ & MILLIER 1971; WHEELER *et al.* 1976), as well as physiological traits (DE PHILIPPIS 1937), is relatively high. Significant differences among provenances were also found by means of terpenic markers (ARBEZ *et al.* 1974; FINESCHI & GROSSONI 1981; PACI *et al.* 1989).

A relatively high differentiation among the "small range species" – or subspecies – of this collective species, and among some populations within subspecies, was observed also by means of isozyme markers (FINESCHI 1983, 1984; NIKOLIĆ & TUCIĆ 1983; SCALT-SOYIANNES *et al.* 1994).

In Central Italy, there is a small population of black pine called "Villetta Barrea black pine" that FUKAREK (1958) considers to be a subspecies of the Austrian pine (*Pinus nigricans* Host. ssp. *italica* Hosschstett.) In several researches carried out by means of different
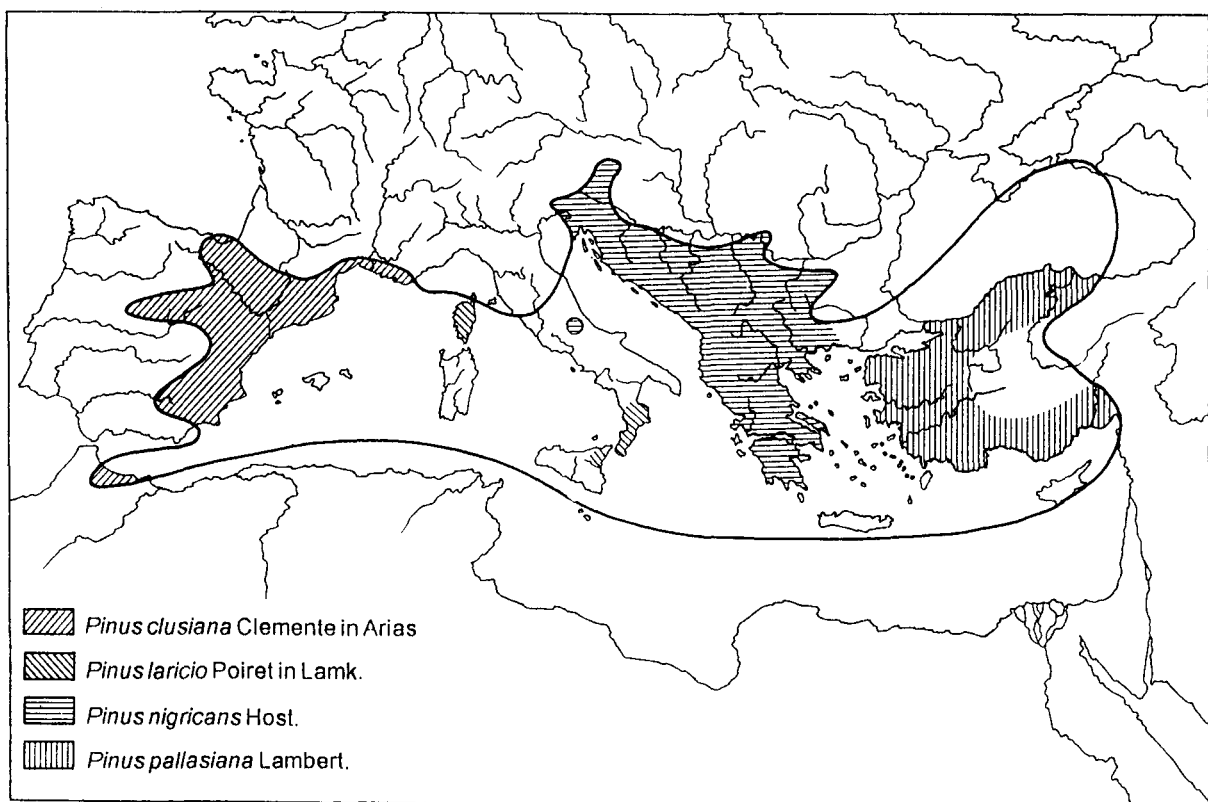

Pinus clusiana Clemente in Arias

Pinus laricio Poiret in Lamk.

Pinus nigricans Host.

Pinus pallasiana Lambert.

Figure 6 Natural range of *Pinus nigra* Arn. (after MIROV 1967). The hatched areas mark the natural ranges of different subspecies
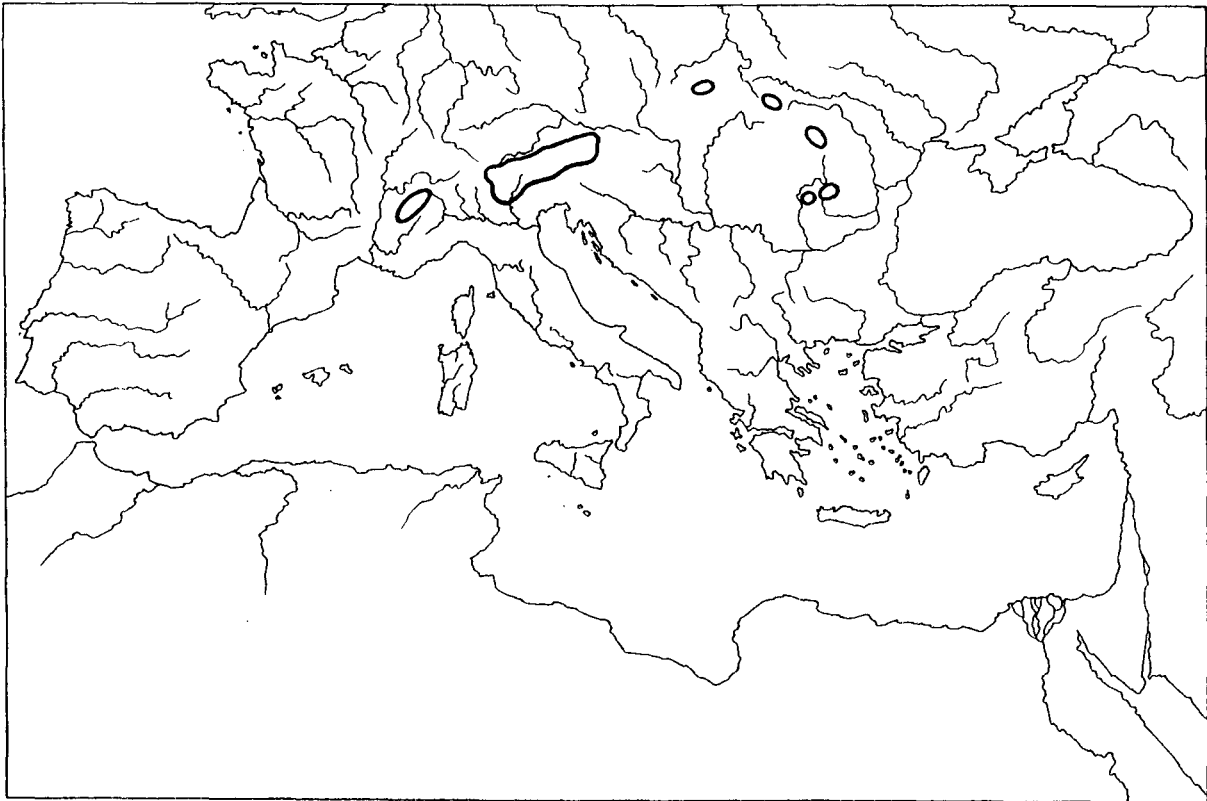
Figure 7 Natural range of *Pinus cembra* Mill. (after MIROV 1967)

markers, the authors confirmed Fukarek's classification; however, they also observed that this subspecies exhibits some intermediate features between the Austrian and the Calabrian pine, *Pinus laricio* Poiret in Lamk. ssp. *calabrica* Delamare (DE PHILIPPIS 1937; GELLINI 1968; FINESCHI 1983, 1984; PACI *et al.* 1989). This important and isolated population is luckily located inside the National Park of Abruzzo.

FINESCHI (1984), by means of the allozyme locus *Skdh-B*, observed that a Tuscan isolated and small group of *P. laricio* could probably be attributed to the Corsican subspecies (ssp. *corsicana* Loud).

Recently, SCALTSOYIANNES *et al.* (1994) have again observed *P. laricio* (Corsican and Calabrian) to be clearly distinct from *P. nigricans*.

The discontinuity of the black pine geographic range and the sometimes marked differentiation among taxonomic entities and populations make this species very rich in genetic resources that need appropriate protection and, therefore, a greater research effort for the future.

Stone pine

Pinus cembra has a discontinuous range mainly in the Alpine region, separated into relatively small and isolated zones (Fig. 7). SZMIDT (1982) studied 10 populations distributed inside this range and an Asiatic

one – which is attributed by the author to the same species, but belongs to the closely related species *Pinus sibirica* Du Tour (KRUTOVSKII *et al.*, 1990) – and found a very high differentiation among some of them, especially in a Romanian isolated population and in the Asiatic one, as shown by values of genetic distance by NEI (1972); genetic differentiation among populations from Tatra Mts. and the Alps is smaller but still remarkable compared to populations of other conifer species. Values of gene diversity within populations (expected heterozygosity) are similar to those found in most conifers.

This species too would deserve being studied to a greater extent in order to protect forest genetic resources, because of differentiation among populations scattered in a discontinuous natural range, whose intrapopulation genetic variation is not low.

Aleppo pine

Pinus halepensis Mill. is the most widely distributed pine of the Mediterranean region (Fig. 8). Using isozymes, SCHILLER *et al.* (1986) studied 19 populations representative of its natural range (most of them had been sampled for an international FAO research project on provenances of Aleppo pine and *Pinus brutia* Ten.); they found low values of genetic diversity within populations, but were able to subdivide them into two
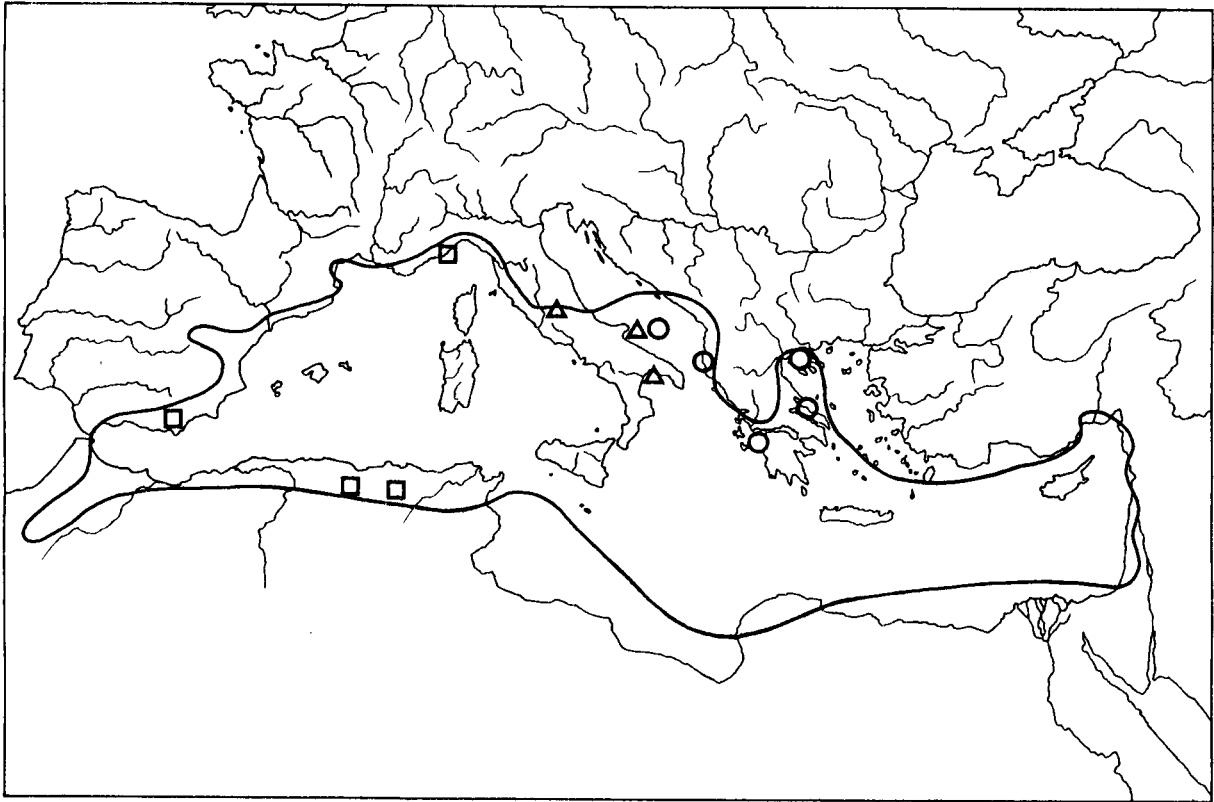
Figure 8 Natural range of *Pinus halepensis* Mill. (after MIROV 1967). Squares, triangles and circles point to the populations, belonging to three differentiated groups, studied by CALAMASSI *et al.* (1988). In particular, circles point to populations belonging to the "eastern European race", differentiated and intrgressed with alleles from *Pinus brutia* Ten., found out by SCHILLER *et al.* (1986) using isozymes. The Albanian population was studied only by the latter. The population from Gargano (Southern Italy) is marked by two symbols because it was differently classified in the quoted papers (see text).

groups: western and eastern Mediterranean. The former is subdivided into four races, of which one, that from Eastern Europe, consists of populations introgressed with alleles from *P. brutia* therefore exhibiting higher values of genetic variation within populations: Southern Italy (Gargano, Puglia), Albania, and Greece (an introgressed population from Libya shows low heterozygosity, but the analysed seed sample was collected from a small number of trees, so the authors classified it as belonging to the eastern European race only tentatively). These populations would be worthy of protection.

CALAMASSI *et al.* (1988) studied some anatomical and morphological needle traits on samples picked from plants grown in a plantation close to Florence, which was established using seeds collected in the species natural range for the above-mentioned FAO project; some sampled populations were the same as those studied by SCHILLER *et al.* (1986). Results obtained by different multivariate analysis methods showed a sharp subdivision of the ten investigated populations into three well defined groups: populations from Greece, from Central and Southern Italy, and from the western

sector of the range that includes Northern Italy, Spain and Algeria. It is of interest to note that some populations, common to both researches, were differently grouped as a result of using different markers (Fig. 8).

Using terpene markers, SCHILLER and GRUNWALD (1987) observed a very low differentiation among populations, even though they were able to group them in three distinct clusters (Greece, Western Europe and North Africa), which are similar to those observed by CALAMASSI *et al.* (1988) using morphological traits. BARADAT *et al.* (1989), analyzing terpene composition of four Italian natural populations, could clearly distinguish the population from Central Italy, and partly the one from Northern Italy, from the rest.

Significant differences among provenances were found for germination and resistance to water stress of seeds and seedlings (CALAMASSI *et al.* 1980; FALUSI *et al.* 1983).

In conclusion, since this is an important species for Mediterranean countries, further studies may be useful to improve knowledge of the genetic structure of the more differentiated populations (the introgressed populations belonging to the eastern European race found by SCHILLER *et al.*, 1986), and to find, elsewhere

specific genetic information, if any, in order to include them in genetic resource conservation programs.

CONCLUSIONS

The above-mentioned examples are partial and only given for indication, but they help showing that gene conservation in pine species requires:

a) a greater effort in research on population genetics of these species;

b) an integration of results obtained by means of different markers. A work of synthesis may be important in order to indicate gene resources to be protected under gene conservation programs.

Specific features of forest tree species and the environmental condition of present-day world suggest, in particular, forms of dynamic conservation *in situ*, in order to preserve the genetic adaptability of tree populations under conditions of global environmental change. This kind of conservation allows populations to evolve and therefore to preserve their genetic variation, which originates from mutation, migration (gene flow) and sexual reproduction, by means of a continuous adaptation to spatial and temporal heterogeneity of environmental conditions. It involves the presence of a genetic load, that is less fit genetic information which provides the basis for adaptation to future environmental changes; it is reduced by selection, so it cannot become excessive lowering population adaptability (GREGORIUS 1989, 1991; ZIEHE *et al.*, 1989).

Sometimes it may be necessary to match physical protection measures of stands with suitable sylvicultural treatments, either when natural regeneration is scanty or in order to prevent the settlement of different species under the adult trees, as it often happens for instance in Aleppo pine stands and in Scots pine Mediterranean populations. This requirement involves also sylvicultural research on natural regeneration (ZIEHE *et al.* 1989).

Measures of dynamic conservation *in situ* applied to minor and isolated relict populations – like stone pine in several zones of its range, *Pinus leucodermis* in Southern Italy and in some zones of its Balkanic range (whose gene resources need to be more studied), black pine in Central Italy and Scots pine in the Northern Apennines and in Scotland – should be matched with some appropriate forms of dynamic conservation *ex situ*, in order to ensure preservation of their gene resources in case adult trees should disappear altogether without any natural regeneration, as – for instance – following extended and recurrent fires, which are so common in Mediterranean regions. As a matter of fact, experimental plots and reforestations of *Pinus leuco- dermis*, established in Southern Italy in the neighbour- hood of some natural populations and using locally collected seeds, can be useful for gene conservation, just as are provenance plantations for other species (Scots pine, black pine and Aleppo pine, for instance).

Forms of static conservation are hardly applicable to forest tree species because of their biological features – especially generation length and reproductive system – and could lead to the accumulation of an excessive genetic load or to gene loss due to drift effects, and therefore to lower adaptability. Nevertheless, in some particular cases and in situations of emergency, conser- vation of seeds or other reproductive material may be very important, but such solutions cannot be applied on a large scale and in any case such materials should be regenerated as soon as possible (GREGORIUS 1989, 1991; ZIEHE *et al.* 1989).

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